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# IS THERE A TRANSFORMATION OF SEX IN FROGS?

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THIS paper is a reply to the recent article of Dr. Emil Witschi which appeared in a late issue of the NATURALIST (Vol. LV, No. 641). Witschi is quite convinced that the problem of sex development and differentiation in frogs has been settled, and that nothing further remains to be said. However, the writer feels that instead of being solved, the time has come for a revision of the entire question of sex development in Anurans, and that the subject is ripe for a reinterpretation upon a more rational basis than that accorded to it heretofore.

The first portion of the paper will be devoted to a brief exposition of the writer's interpretation of sex in frog larvæ based upon data obtained from a study of the bullfrog. The second part of the paper is a reply to certain questions raised by Dr. Witschi.

In larval males of the bullfrog two gonads are formed, just as there are two kidneys formed, a pro-testis or embryonic sex gland destined to degenerate and disappear in ontogenetic development and a definite or functional testis which replaces it. The germinal elements of the pro-testis arise in the entoderm and migrate into the germ ridges early in embryonic life. The cells multiply rapidly and together with the mesodermal elements of the germ glands form paired ridges projecting into the cœlomic cavity. While the tadpole is very immature and has yet a year of larval life before metamorphosing, the

germ cells of the pro-testis undergo a precocious and abortive sexual cycle culminating in degeneration and resorption. Beautiful cysts of spermatocytes are formed, but the first maturation division rarely proceeds past the

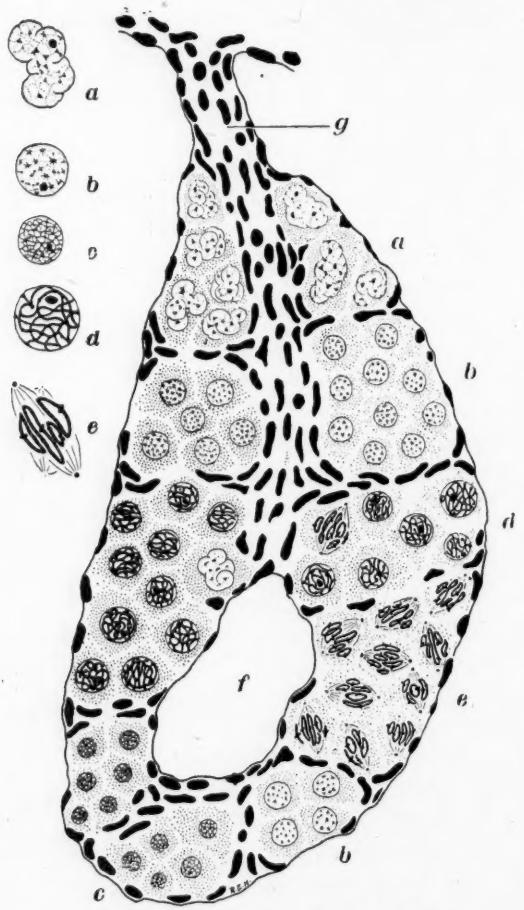


FIG. 1. Transverse section pro-testis *R. catesbeiana* tadpole. Animal has a year of larval life remaining. *A*, Spermatogonia showing nuclear polymorphism due to incomplete fusion chromosomal vesicles; *B*, Final spermatogonia; *C*, Spermatocytes in leptotene stage; *D*, Amphitene and pachytene; *E*, Heterotypic mitosis; *F*, Secondary genital cavity; *G*, Anlage of sex cords of definitive gonad which develops as a core within pro-testis.

anaphase owing to fragmentation of the centrosome and consequent formation of polyasters (Fig. 1). Sometimes aberrant spermatids are formed by suppression of the first and second maturation divisions and growth of axial filaments from the centrosome. Practically all the germ cells of the pro-testis degenerate and disappear while in various stages of maturation—some undergo an oviform type of degeneration, *i.e.*, hypertrophy enormously and take on the superficial characters of oocytes. The oviform type of degeneration, however, is more characteristic of the short larval-lived frogs than of *R. catesbeiana*, for in many animals these large cells appear rarely and in others not at all and this is an important point to keep in mind. This type of degeneration will be discussed in detail in a later paper; suffice to say it gives no clue to the sex of a cell. (See Plates 1 and 2.)

Some cells of the pro-testis fail to take part in the abortive sexual cycle persisting through the phase of maturation and degenerate as spermatogonia. These elements migrate into the sex cords (Fig. 1, *g*) which have formed meantime, and form a core of germinal tissue extending through the center of the pro-testis. This core of tissue plus the sex cords is the anlage of the definitive testis and is quite distinct from the pro-testis, the cells of which are maturating and degenerating, whereas the cells of the forming functional gonad remain as primitive spermatogonia. The definitive testis by rapid growth completely supplants the pro-testis which usually disappears some time before metamorphosis. The functional gonad is generally fully formed at metamorphosis when the larvæ are two years of age. Some tadpoles, but not all, develop ripe spermatozoa in the gonad at metamorphosis due to a second sexual cycle of the germ cells of the definitive gonad. (Swingle, '21, *Jour. Exp. Zool.*, Vol. 32.)

In the frogs with short larval-life the same succession of gonads occurs, but in these forms the developmental processes are greatly accelerated and the pro-testis ma-

turation cycle is cut short by the cells early becoming senescent and undergoing oviform degeneration *i.e.*, hypertrophy to such an extent as to superficially resemble oocytes. This oviform degeneration occurs to an even more marked degree in the progonad of the toad which has a still shorter larval-life, *e.g.*, in Bidder's organ. In male anurans the entire pro-testis or larval gonad is the homologue of the male organ of Bidder in *Bufo*.

The pro-testis of the short larval-lived frogs has been misinterpreted as an ovary owing to the oviform-type of degeneration characteristic of many of its senescent cells, and hence tadpoles are said to develop first as females, fifty per cent. later transforming into males. The normal embryological process by which the definitive testis develops as a central axis through the degenerating pro-testis or larval Bidder's organ, has been described by Witschi as the transformation of female tadpoles into males. In *R. catesbeiana,* where the larval life is prolonged over two years, the true nature of the pro-testis is revealed, for relatively few of the cells are of the oviform type and all transition stages between such cells and normal spermatocytes occur. The evidence presented by this material will be published in due time, and is too clean-cut to admit of any doubt that the entire larval gonad of male anurans is simply an embryonic male sex gland rudiment and not a temporary ovary. Witschi's Fig. 6 (this journal, Vol. LV), which he supposes is an ovary transforming into a testis is simply a transition stage in the development of the definitive testis, and degeneration of the pro-testis or Bidder's organ in a short larval-lived frog. Compare his Fig. 6 with Fig. 1 of this paper and note how the true male character of the cells of the pro-testis comes out in *Rana catesbeiana* tadpoles.

When the facts are considered it is evident that the transitory gonadic rudiment of male frog larvæ is an organ of Bidder which degenerates and is replaced by the definitive gonad. Any one who has studied the oviform-like

cells of the so-called sexually intermediate tadpoles and compared them with the cells of Bidder's organ in male toads, is at once struck by the remarkable similarity in their origin, development, structure and fate in the two groups. They are identical. The crux of the problem is the nature of Bidder's organ in male Bufonidæ and of the oviform-like cells of the pro-testis. The advocates of sex transformation have assumed that such cells are undoubtedly female, but no proof has ever been advanced that they are. Their ultimate fate is the same as that of the first year spermatocytes in the bullfrog tadpole — degeneration (see Plates 1 and 2). The sex-transformationists have been misled by the idea that everything superficially resembling an oocyte is necessarily such, or that any cell in tadpoles and first-year animals undergoing the early growth stages, leptotene, pachytene, etc., is to be regarded as female. These are fallacious criteria. Enormously hypertrophied oocyte-like cells which have passed through the early growth stages and entered the "germinal vesicle" period so characteristic of oocytes, occur as normal features of the male sexual cycle of certain animals, *e.g.*, myriapods (Figs. 5–8). These animals were at first regarded as hermaphrodites by Blackman (1905, *Bull. Mus. Comp. Zool.*, Harvard, Vol. XLVIII, no. 1) who found upon examination, however, that these "oocytes" were in reality spermatocytes of giant proportions, and developed into spermatozoa. The writer has examined some of Professor Blackman's material and the oocyte-like character of the male sex-cells is remarkable. In the material examined these cells practically fill the gonads. Firket, 1920, working on the chick embryo, describes and figures spermatocytes undergoing oviform degeneration, *i.e.*, enlarging to such an extent as to resemble oocytes. There are many other cases reported in the literature. How does Witschi know that the transitory oocyte-like cells he describes in the future male tadpoles or so-called hermaphrodites, are female cells and not senescent organ of Bidder cells occurring

in the course of the abortive and degenerate sexual cycle of an embryonic pro-testis?

The work of Witschi on the problem of sex in anurans can be summarized thus: He has described in great detail and with admirable exactness the process of development of the pro-testis or Bidder's organ in the short larval-lived frogs, its degeneration and final replacement by the definitive gonad. This process he calls transformation of females into males. The experimental investigations of Witschi upon sex transformation by environmental influences consists of this: By means of such agents as heat or cold, etc., he has simply modified the normal course of development of the pro-testis — Bidder's organ, thereby accelerating or delaying the development of the definitive testis. The experimental results show that it is possible to modify the developmental rate of the embryonic testis. Similar experiments carried out with regard to other larval structures would unquestionably give similar developmental modification. Cold hinders metamorphosis and all the normal structural changes metamorphosis implies. All of these environmental influences are interferences with the normal cycle of the gonads, by which the development of the definitive gonad out of the pro-testis is accelerated, retarded, or possibly prevented entirely. The following quotation from Witschi '14, page 10, is significant in this connection:

> Bei seinen Untersuchungen war es Hertwig aufgefallen, das unter dem Einfluss verschiedener Aussenbedingungen sich nicht nur die Geschlechtsziffern, sondern oft auch in ganz auffälliger Weise der Rhythmus, in Welchem die Keimdrüsen und manche andere Organe sich anlegen und entwickeln.

It is probable, judging from certain experiments reported, that the degree of development attained by the larval gonadic rudiment, its position in relation to the definitive gonads, its period of persistence, non-formation in some forms, and such like, may vary in different frog species and is determined by heritable factors. For example, in *Bufo*, the structure persists throughout life in

males, disappears after two years in females, and is anterior to the functional gonads. In frogs it forms the outer husk of the germ gland enclosing the centrally developing functional testis and may or may not show the oviform type of degeneration, *e.g., R. catesbeiana.*

If sex is so labile in tadpoles and young frogs, and females so readily transform into males under environmental stimuli, why is it that such sex reversals do not occur in adult frogs *after the degeneration of the protestis* and the formation of the definitive testis has occurred? All investigators are agreed that the sex ratio of adult frogs of all species reported is approximately 50 – 50. If environment (ever changing in the same locality, and never the same in different regions), plays such an important sex transforming rôle, why do male tadpoles never transform into females — all investigators agree that they do not. Why do only fifty per cent. of the so-called larval females transform into males if they were not zygotic males from the beginning, and why do not all female frog larvæ transform into males instead of only fifty per cent. if such transformation is possible? Appeal cannot be made to Professor Hertwig's well-known late fertilization experiments because in these experiments the influence of the over-ripeness of the egg upon the zygotic conditions determining sex are unknown. Hormones! To date there is no positive evidence that such secretions have ever actually changed a female germ cell into a functional male germ-cell.

Cases of hermaphroditism in adult frogs are thought by some to furnish evidence of a sex transformation in frogs. However, true hermaphroditism in adult frogs is as rare a phenomenon as it is in mammals when we consider the few recorded cases, and the enormous number of frogs annually dissected the world over. Crew ('21), *Journal of Genetics,* Vol. II, no. 2, has summarized the recorded cases of abnormal sexual organs in frogs and states that there are forty cases. To this number should be added a recent case described in the bullfrog, making

forty-one. Among these forty-one cases, there are but twenty-seven true hermaphrodites. Crew's cases, twenty-one to thirty-three, inclusive, are not hermaphrodites, nor is case thirty-eight, as none of the animals possess ovotestes and some are entirely without gonads. True hermaphroditism in frogs is a permanent and pathological condition, probably due to a mix-up in the genetic constitution of the individual, and is not to be confused with the present problem which has to do with a normal but transitory embryological process.

Much has been written about the marked " sex potencies " of various portions of the gonads in so-called sexually intermediate frogs, *i.e.,* females transforming into males. It is claimed that the outer rind of the gonad exerts a profound female sex influence, while the inner portion exerts a purely male influence. Germ-cells remaining in the outer husk (the main portion of the larval gonad by the way) of the gland are female, those migrating into the central part among the sex cords become male. All such speculations are based upon misinterpretations. The outer portion or husk of the larval male gonad is simply the pro-testis, the cells of which are undergoing a precocious maturation cycle just as they do in the organ of Bidder in *Bufo,* the inner portion or sex cord region is where the definitive gonad begins development and as it spreads and grows the embryonic male gonad degenerates and disappears. *It is in the region of most marked " female " tendencies that the writer finds in the bullfrog entire cysts of unmistakable spermatocytes, and occasional spermatids* (Fig. 1, *e*). In other words, the pro-testis — what Witschi regards as an ovary — can in the bullfrog, where its development is greatly prolonged, give rise to practically mature male sex products. Recently, the writer made an observation of considerable interest. In the degenerating Bidder's organ (pro-testis) of a two-year-old male larva in which formation of the definitive testis had been delayed until metamorphosis and in which the oviform type of degeneration

was the most marked of any animal yet observed, several cysts of unmistakable spermatocytes and spermatids were observed. They arose from the maturating cells of what Witschi regards as the female part of the gonad — in reality the pro-testis, and were of the cell type characteristic of the adult frog. This observation shows two things clearly: (1) *That the direct descendants of the male primordial germ cells (pro-testis elements) can produce practically mature germ cells;* (2) *that the spermatocytes of the structure regarded by the writer as a pro-testis are really male cells, and that the structure in so-called sexually intermediate frogs and tadpoles is in no sense to be regarded as female in character.*

Another point is of interest here — the writer has never observed direct testicular development in *R. catesbeiana,* though it probably occurs in some strains; the indirect method alone has been found, *e.g.,* first a pro-testis is formed which is later supplanted by the definitive gonad. In the bullfrog, which has the longest larval life of any anuran, the pro-testis persists longer than in other forms, sometimes two years before giving place to the definitive gonad. What the writer calls a pro-testis of so-called sexually intermediate tadpoles is according to Witschi a transitory ovary. If this is true why is it that despite its persistence for such a long time, relatively few oocyte-like cells are found in *R. catesbeiana* and in many individuals none, throughout a two-year period, but instead the structure produces spermatocytes and sometimes spermatids? Why is it, if this structure is an ovary in the so-called females that later transform into males, that the shorter the larval life of male anurans, the more the pro-testis in its structure and behavior resembles the Bidder's organ characteristic of male toads, due to rapid oviform degeneration of its cells; the longer the larval life, *e.g., Rana catesbeiana,* the more the germinal elements undergo a normal sexual cycle characteristic of male cells? The answer is, because in forms with extraordinary prolonged larval lives the

true nature of the embryonic male gonad has sufficient time to manifest itself before being supplanted by the definitive gland.

We come now to a discussion of the nature of Bidder's organ in *Bufo*, for this is the classical example of oviform degeneration of racially senescent germ cells. Heretofore, this embryonic sex gland rudiment has been regarded as characteristic of toads, but such is not the case. In frogs the pro-testis or larval gonad is a Bidder's organ, destined to be replaced by the definitive male gonad developing within; in male toad larvæ on the other hand, the functional gonad arises behind the pro-testis or Bidder's organ, consequently this structure persists as a degenerate gonadic rudiment attached to the functional gland.

According to the writer's view, Bidder's organ in *Bufo* is simply a vestigial larval gonad persisting throughout life and has the same sex as the definitive gonad behind it — male in males, female in females. It is just as though the pro-nephros of tadpoles persisted as a nonfunctional and degenerate rudiment at the end of the mesonephros. That many such larval and embryonic rudiments do persist through adult life in various animals is a commonplace of embryology, and their persistence in one species and total disappearance in another related one, is also well known. Bidder's organ in *Bufo* then, is a persisting, in frogs a transitory, embryonic sex gland rudiment, a relic of a phylogenetically earlier sexual condition. The functional gonads are more recently acquired structures (like the larval mesonephros) superimposed upon the older degenerate glands. Briefly stated, the evidence for the view that Bidder's organ is homologous to the pro-testis of frogs and that it is not a rudimentary ovary except in female animals is as follows:

1. The cells of Bidder's organ in *Bufo* are unquestionably germ cells. The gland appears very early in embryonic life (two weeks after hatching) and its cells far

outstrip in development the cells of the definitive gonads located posteriorly.

2. The cells of Bidder's organ extremely early in development undergo a precocious and abortive maturation cycle and become senescent and degenerate oocyte-like structures when the germinal elements of the functional gonads have barely started to multiply to form the definitive glands. This occurs in individuals of both sexes.

3. The larval maturation cycle such as occurs in the bullfrog, and in other anurans, throughout the entire larval gonad is confined to Bidder's organ in *Bufo,* and the changes occurring in this structure do not affect the normal developmental cycle of the definitive germ glands behind.

4. The so-called transformation of female animals into males, claimed by Witschi and others to be the normal course of development in frogs, does not occur in toads. Why? Because in *Bufo,* the definitive gonads are from the beginning located posterior to Bidder's organ, and it is not necessary in order that they may develop that this structure degenerate and disappear as is the case in frogs where the definitive testis starts development as a core within the pro-testis or Bidder's organ, necessitating its complete destruction.

5. Few have ever claimed that sex in toads is labile and easily reversed by environmental influences. Why? Because the sex of the definitive gonads is definitely fixed and clear cut at an early stage of life. The separation of Bidder's organ and the gonads has precluded the possibility of confusing the pro-gonad and the definitive gonad.

6. Bidder's organ is merely a persisting embryonic gonad whose cells have undergone oviform degeneration. It is not a rudimentary ovary except in female animals. This is indicated by its presence in both sexes in toads; its presence in Spengel's case of true hermaphroditism; by the fact that neither in male or female of toads do

its cells develop into true functional eggs; and by its degenerate structure from its inception in both sexes.

In a recent paper (*Zoologischer Anzeiger,* Dec., 1921) Harms describes marked hypertrophy of Bidder's organ following testis removal. He considers that castration of males causes Bidder's organ to develop into an ovary. However, it should be noted that such operated animals with hypertrophied Bidder's organ (ovary according to Harms) retain all their male secondary sex characters, and their normal mating instincts and that these male characters and instincts undergo a normal cyclical development in such induced "females." When Harms removed both testes and Bidder's organ the somatic sex characters and instincts failed to develop, showing clearly that Bidder's organ in male toads acts like a testis in maintaining the secondary sexual characters. This is excellent evidence for the writer's view that in male toads Bidder's organ is simply a persisting embryonic male sex gland rudiment and not an ovary. If it is an ovary why should it develop and maintain the secondary sex characters of the male in absence of the testis?

7. Recent investigators have inclined to the view that this structure is a hermaphrodite gland, *i.e.,* in male toads a rudimentary ovary, in females a rudimentary testis. If this is true then the admission is made that large, senescent, oocyte-like germ cells are not necessarily female cells — the crucial point for which the writer is contending.

8. Bidder's organ in *Bufo* corresponds to the larval gonad of frogs which in these forms disappears in the male and is replaced by the definitive testis. In the case of female anurans so far as the writer is aware no one has carried out a thorough investigation of the germ cycle from larval to fully adult life to see whether or not such a degeneration occurs in the female line. In mammals and birds such degeneration of the female embryonic line of germ-cells is quite well established as the work of Winiwarter, Firket and others shows.

The writer is of the opinion that it is only by adopting the view advanced here regarding the homologous nature of the larval male gonad of frogs, and Bidder's organ in *Bufo*, that the problem of sex differentiation in anurans can be placed upon a rational basis. The theory accords with the embryological facts, covers the experimental finding of Witschi and others, accords with our own cytological data in the bullfrog, accords with the embryonic sexual conditions of other vertebrates, *i.e.*, the degeneration of the embryonic line of germ cells in birds and mammals, and lastly furnishes an explanation of Bidder's organ in *Bufo*.

The key to the puzzle of sex development in frogs is simply this: every cell that superficially resembles an oocyte is not necessarily a female cell especially when occurring in an otherwise male individual, and that the larval male gonad of anurans is an organ of Bidder — a rudimentary embryonic sex gland with the same sex as the definitive gonad arising out of it. Misinterpretation of oviform hypertrophy and degeneration of racially senescent sex cells has rendered chaotic the problem of sex differentiation in anurans (see Plates 1 and 2).

Witschi regards the development of certain somatic sex characters such as the Müllerian ducts as very positive evidence for his theory of sex transformation. He says:

> In males which show a typical development of the testicles, no Müllerian ducts of any significance are formed. On the other hand, such animals as first develop ovaries and later undergo the transformation of sex, also show regular oviducts; and these continue to grow just up to the time when the transformation of sex begins. This parallelism in the behavior of the Müllerian ducts and the gonads furnishes definite proof that the "eggs" and "ovocytes," described by the writer, are in fact really eggs and ovocytes and that the transformation of sex is a well-established fact. After the transformation of sex, when the ovocytes have disappeared, the Müllerian ducts begin to shrink but they do not disappear completely, etc., etc.

The following data shows that in reality such so-called parallelism in the behavior of the Müllerian ducts and

the gonads does not exist and that evidence based on such parallelism is worthless.

In the normal males of adult *Rana pipiens* the Müllerian ducts are remarkable for their size and degree of development. They arise as cellular cords in the peritoneum at the time of metamorphosis and only acquire full development long after transformation when they come to resemble to a striking degree the oviducts of females (Fig. 2). In the larva of *R. pipiens* the so-called

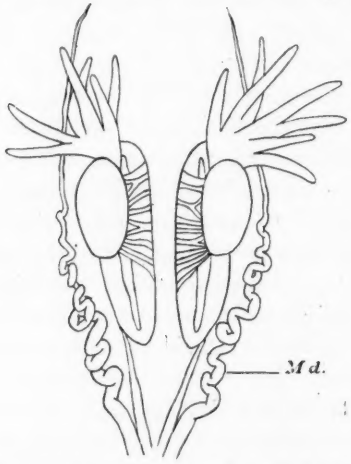


FIG. 2. Urogenital apparatus of adult *Rana pipiens* showing the normal condition of the Müllerian ducts (*md*) in males of this species.

transformation of females into males (degeneration of the pro-testis and formation of the definitive testis) occurs very early in larval life, before the Müllerian ducts appear, and in this species the ducts undergo practically their entire development after the definitive testis has formed. In other words, while subjected to the influence of the fully formed testis and its ripening sex products the ducts undergo the most marked development known in the males of any anuran species. Moreover, in *Rana catesbeiana*, where if we accept Witschi's interpretation of femaleness, the so-called transformation of female in-

dividuals into males is a prolonged process requiring two years, and where the future male larvæ are subjected to the so-called female influence during the entire period, the Müllerian duct does not appear. At metamorphosis when the definitive testes are fully formed and spermatozoa are beginning to appear the cellular cords representing the vestigial Müllerian ducts of the male form but do not develop. If Witschi's interpretation were correct, one would certainly expect to find marked development and hypertrophy of the Müllerian ducts in *R. catesbeiana* because of their being so long exposed to female influence. As a matter of fact, these structures in the male bullfrog are less developed than in other forms.

The same criticism applies to the so-called developmental correlation of the Müllerian duct with the gonad of the same side in cases of lateral hermaphroditism. What Witschi terms lateral hermaphrodites are nothing more than larvæ or young frogs which show the definitive testis developing out of the pro-testis (larval male Bidder's organ) faster on one side than on the other. (See Witschi, Am. Nat., page 533.) In the end such animals develop into definite males with testes symmetrically formed. True lateral hermaphroditism in adult frogs is an exceedingly rare phenomenon. In the writer's material it is rare to find both definitive testes developing out of the pro-testes at the same rate, one gland may be the finished gonad, the other the pro-testis undergoing degeneration. Such larvæ are in no sense to be regarded as lateral hermaphrodites. There is no developmental correlation of the Müllerian ducts with the gonad of the same side in *R. catesbeiana* and *R. pipiens*, because there are no ducts formed until after the definitive testes are formed. Regarding the other somatic sex characters such as seminal vesicles and thumb cushions, it should be pointed out that the thumb pad in *R. catesbeiana* is not formed until after metamorphosis when

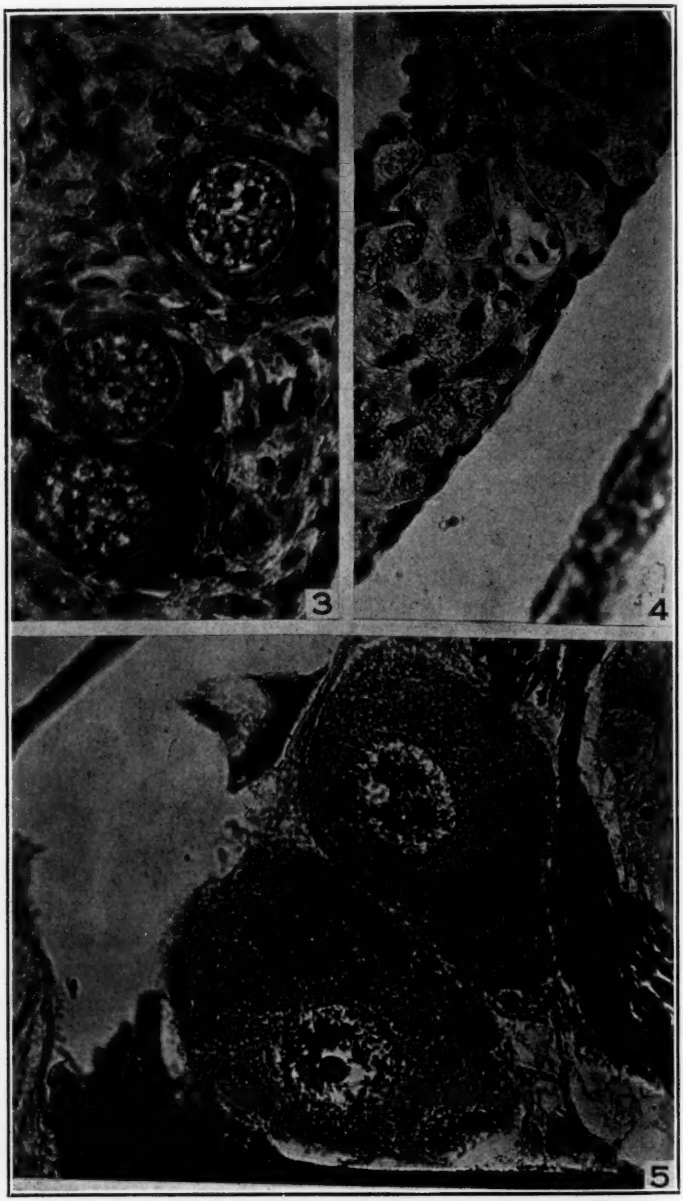


PLATE I

FIG. 3. So-called oocytes occurring in the degenerating pro-testis of larval bullfrogs. These cells according to the writer's view are merely hypertrophied spermatocytes that have undergone oviform degeneration.

FIG. 4. Section of pro-testis male larva before onset of degeneration. At *X* is spermatocyte in prophase. The black bodies are ring tetrads.

FIG. 5. The giant spermatocytes of *Scolopendra Heros* (Chilopoda). These cells form functional spermatozoa and make up the greater part of the testes. Note the "germinal vesicle" condition of the nucleus.

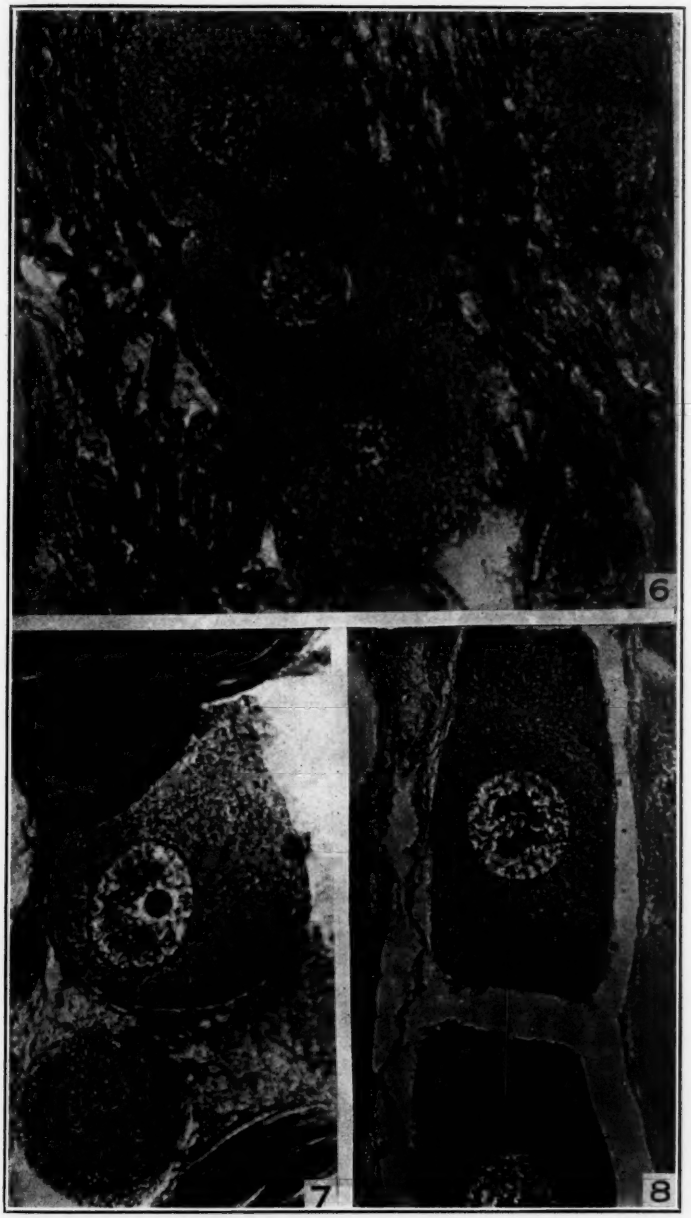


PLATE II

FIG. 6. Spermatocytes of *Scolopendra*.

FIGS. 7 and 8. Spermatocytes of *Lithobius* (Chilopoda). The resemblance to oocytes in the germinal vesicle stage is remarkable. Sections of the testes look like ovaries.

All photographs on Plates I and II made at a magnification of 500 diameters. No reduction. Figures 5–8 are from Professor Blackman's material.

the fully formed testes are present, and the seminal vesicles are absent or rudimentary in the males of many frog species, and exceedingly well developed in others.

In the few cases reported of true lateral hermaphroditism in adult frogs there is *not always* a developmental correlation of the Müllerian ducts with the gonad of the same side. Crew ('21), (*Journal of Genetics,* Vol. II, no. 2) has summarized the known cases of sexual abnormality in amphibians — see Figs. 7, 8, 9, 12, 14, and 16 of this paper, also the report of cases 21, 22, 23, 24, and 39. These are exceptions to any rule of developmental correlation. In several cases, Figs. 25 and 31, the ducts are quite as well developed in total absence of ovarian tissue as when such is present in large amounts, this, of course, being the normal condition in *Rana pipiens.* Crew also gives a list of frog cases reported where both gonads were entirely missing and yet the Müllerian ducts were well developed in such individuals.

Because of these facts it is fair to conclude that the appeal to the somatic sex characters completely fails as proof of the transformation of female frogs into males.

In closing, it should be pointed out that Witschi has made but one original investigation of sex in anurans (Witschi '14, no. 1). His later papers on the subject contain no new observations or experiments but are purely speculative endeavors to interpret his early work in accordance with Mendelism ('14, no. 2), later ('20, no. 3) in accordance with internal secretions.

# THE SEX-LINKED GROUP OF MUTANT CHARACTERS IN DROSOPHILA WILLISTONI

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## INTRODUCTION

THE present work was undertaken for the purpose of comparing the genetical behavior of the fruit-fly *Drosophila willistoni* with that of *Drosophila melanogaster* and other members of the genus. It deals with the 28 sex-linked mutant characters thus far studied. The non-sex-linked characters will be considered in another paper.

*Drosophila willistoni* Sturtevant (*D. pallida* Williston)[1] is not unlike the well-known *D. melanogaster* in habits and superficial appearance, but a detailed examination reveals numerous features in which it differs from *melanogaster*. Among these are the following: (1) absence of sex combs in the male, (2) six instead of eight rows of acrostichal hairs on the thorax, (3) smaller size and more slender form, (4) vermilion instead of red eye color, and (5) narrow instead of broad bands on the abdomen.

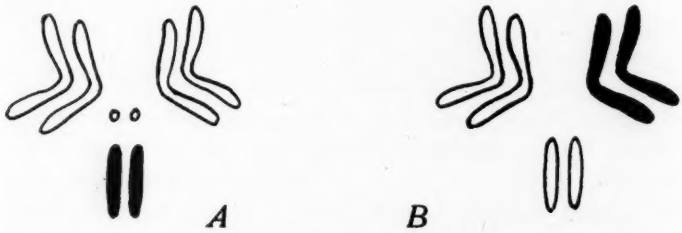
This species has been chosen for the present study because it is one of the species having the same general type of chromosome group as *D. melanogaster*. It will be recalled that within the genus Drosophila at least eleven different types of chromosome groups are represented (Metz, 1916). The most common type is that called type A (Fig. *A*, present paper), which is found in 13 of the 29 species studied. In these 13 species (which include *melanogaster* and *willistoni*), the chromosome groups are so much alike as to suggest that similar chromosomes are homologous and carry homologous groups of genes throughout. On the other hand, the species themselves do not form a restricted taxonomic group, but seem to be

[1] See Sturtevant, 1921*b*, for description, etc.

scattered more or less at random through the genus — which does not conform to such a view unless this type of chromosome group be considered primitive and the forerunner of several other types.

These considerations indicated the need of a comparative study of different species possessing this type of chromosome group, in addition to the studies already being made on species having different types of groups. Since the species can not be hybridized (or have thus far refused to hybridize — with one exception considered below), it is necessary to make cytological and genetical studies of them individually. This of course limits the comparison to a very few species.

The present paper supplements our previous one (Lancefield and Metz, 1921) on the sex chromosome relationships of *willistoni* and *melanogaster,* in which it was shown by means of non-disjunctional flies that the sex chromosomes are not strictly homologous in the two species. In *melanogaster* the short, rod-like pair is the sex chromosome pair (Bridges, 1916), whereas in *willistoni* we find that the rod-like pair is an autosome pair, and that one of the large V-shaped pairs is the sex chromosome pair. This relationship is shown in Figs. *A* and *B*.




FIGS. *A* and *B*. Diagrams of female chromosome groups of *Drosophila melanogaster* and *Drosophila willistoni* respectively. The X-chromosomes are represented in solid black, the autosomes in outline. In *D. willistoni* the small, dot-like pair may be absent.

The genetical study considered here is for the purpose of comparing the constitution of the sex chromosomes by

means of the sex-linked characters and their linkage relations.

The stock of *D. willistoni* which we have used was brought from Cuba in 1915, and was kept in the laboratory without being studied until 1919 when the present work began.

In making the tests for linkage and in calculating crossover values, the usual procedure has been followed.[2] We have been concerned particularly with determining the relative order of the genes and the approximate amount of crossing over between them, but not with obtaining exact crossover values. In consequence, the "chromosome map" given here is to be considered as indicating only the approximate location of the respective genes.

In presenting the data, the mutant types are described, not in chronological order, but in such a way as to follow the serial order of the genes on the chromosome map. All of the sex-linked characters are recessive. The data on the origin of mutants are necessarily imperfect, and in some cases are very meager, owing to the fact that many of the mutants appeared in stock cultures, mass cultures, etc., for which no complete records were taken. In such cases the available data are given briefly under the appropriate headings.

We are indebted to Mr. D. E. Lancefield for carrying out some of the early experiments, and for finding the mutants "rimmed" and "nicked." Similarly, we are indebted to Miss Ruth Ferry for the mutant "yellow" and for carrying out the experiments involving "yellow." To Dr. A. H. Sturtevant we owe many valuable suggestions regarding the comparison of mutant characters in *D. willistoni* with those in *D. melanogaster* and *D. simulans*. We are also indebted to the following persons for making the accompanying drawings: Miss Ruth Lincks — Figs. 1, 3, 7, 8; Mrs. D. B. Young — Figs. 2, 4, 5, 6, 17; Miss E. M. Wallace — Fig. 9, and Miss E. D. Mason — Figs. 10 – 16.

[2] This has been described in earlier papers by Morgan and others, and is to be found in current books on genetics.

## DESCRIPTION AND ORIGIN[3] OF MUTANT CHARACTERS

### *Stubby* (*sy*)

*Description.*—Stubby is a bristle character, manifested by all of the thoracic and head bristles (Fig. 2). These are usually shortened, thickened, and somewhat curled, and often are split or forked at the tip. The two posterior scutellar bristles are frequently tightly twisted together and point anteriorly. The short, thick appearance

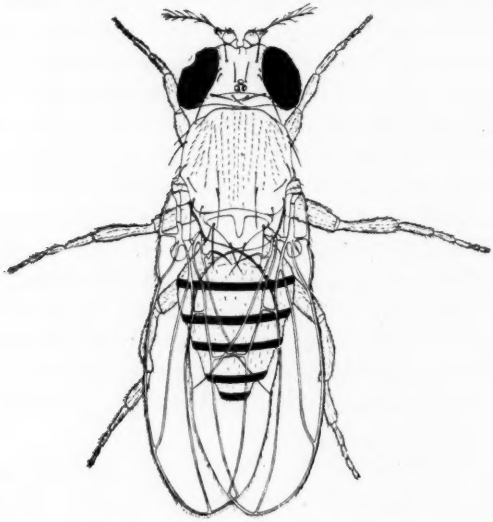


FIG. 1. *Drosophila willistoni*, "normal."

of the bristles is never apparent in combination with small-bristle, but the character can always be distinguished by the forking of the sternopleural bristles. Both sexes are fertile. Stubby looks very much like forked in *melanogaster.*

*Origin.*—One stubby male was obtained from a pair mating. No complete record of this culture was kept, however, and it is not known whether others appeared previously or not.

[3] See Table I.

### *Orange* (*o*)

*Description.*—The eyes are orange colored. In newly hatched flies, the color is a pale lemon, which deepens into orange as the fly matures and may become very dark in old age. The color resembles garnet or coral of *D. melanogaster.*

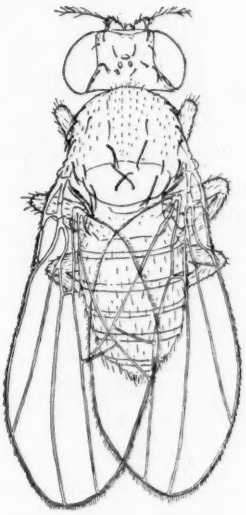


FIG. 2. Stubby, bristles.

*Origin.*—One male appeared among the offspring of a mating of three normal females by an unrelated male. The other offspring were all normal, but their number was not recorded. Presumably, the mutation occurred in one of the $P_1$ females and affected only one or a few germ cells, although it is possible that this female was heterozygous and produced a very small number of the flies in the culture.

### *Small-bristle* (*sb*)

*Description.*—All the bristles are more slender and somewhat shorter than in normal flies. The character is extreme when orange eye color is also present.

*Origin.*—One small-bristle, forked male was obtained from a mass culture carrying rough and forked.

More than a year later, a second mutation to small-bristle occurred in an entirely unrelated stock. In this case the single small-bristle male found among the offspring of one pair was crossed to a female from a homozygous orange small-bristle stock and produced only small-bristle flies.

### *Bent* (*bn*)

*Description.*—The wings of bent flies are slightly spread out, and are bent at the base so that they slope down toward the body (Fig. 3). They are often slightly crumpled. The flies hatch as well as their normal sibs but do not breed as readily.

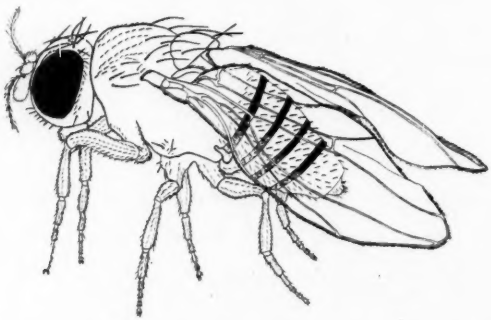


FIG. 3. Bent, wings.

*Origin.*—Many bent males were found in a culture of five orange females from stock mated to a single male of different parentage. At least one of the females carried bent, but the exact origin is uncertain. No bent flies were ever observed in orange stock.

### *Forked* (*f*)

*Description.*—In forked flies, all the bristles are wavy with the ends sometimes forked. The females are sterile. This character is similar to, but less extreme than, stub-

by. It recalls singed$_2$ of *melanogaster* although singed$_2$ is slightly more extreme.

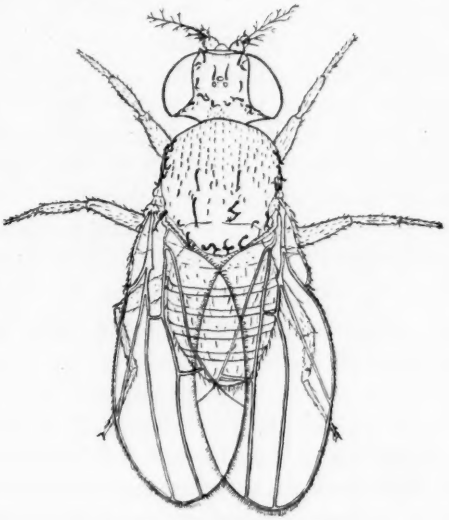


FIG. 4. Forked-2, bristles.

*Origin.*—Seven males were found in a stock bottle. Since no forked females were obtained, it is possible that this was the original appearance of the mutant and that all of the forked flies were from one mother, heterozygous for forked.

*Forked-2* ($f_2$)

*Description.*—This character is much more extreme than its allelomorph, forked, or the similar mutant, stubby. The bristles are twisted and thickened with their ends often split (Fig. 4). The twisting also affects practically all the hairs on the fly, including those on the inner margin of the wing. The hairs on the antennæ are forked. Forked-2 resembles the *melanogaster* singed. The females are sterile.

*Origin.*—Several forked-2 males and one female appeared in a stock bottle of small-bristle flies.

### *Tiny* (*t*)

*Description.*—Tiny-bristle flies usually have small anterior dorso-central bristles. Sometimes the two anterior scutellar bristles are also small. Occasionally all the bristles may be small, so that the individual may be indistinguishable from a true "small-bristle" fly. The character is rather variable and, in many cases, is very hard to separate from normal. This difficulty was so great that the stock was finally discarded.

*Origin.*—A single male was found on the last count of the offspring from a pair mating. It is possible that other tiny males were present among the previous offspring and escaped observation since the character is very inconspicuous.

### *Square* (*sq*)

*Description.*—The wings are about two thirds the normal length with the ends almost square instead of pointed (Fig. 11). A characteristic slight wave extends throughout the length of the wing. The females are sterile and the viability of the males is rather poor.

The description of square suggests that of rudimentary *melanogaster* but square is much less extreme than rudimentary; the wing is not shortened so much and is not cut off so squarely.

*Origin.*—Among the offspring from a pair mating (rough female by orange rough stump male) several square males were found, indicating that the mother was probably heterozygous for square.

### *Reduced* (*re*)

*Description.*—Reduced flies regularly lack the two anterior dorso-central bristles; occasionally, they also lack one of the posterior dorso-centrals; and less frequently, all four are absent. In combination with scute-2, however, the more extreme condition of reduced is frequently found (Fig. 7). The reduced gene also affects the shape

of the abdomen, which is blunted, or apparently compressed along the anterior-posterior axis. The abdominal bands are slightly irregular.

Reduced flies, especially females, are hard to breed in pairs, although those which do produce offspring seem normally fertile.

*Origin.*—Many males were found among the offspring from a pair mating from orange stock. The mother of the culture was apparently heterozygous for the gene.

## The Scute Allelomorphic Series

### 1. *Scute* (*sc*)

*Description.*—The two anterior scutellar bristles are usually lacking, although occasionally only one may be gone. Rarely the combination of one anterior scutellar bristle and one posterior one may be found. The remaining bristles are normal in size. The character almost always manifests itself in homozygous flies. Only one exception to this has been detected up to the present time.

*Origin.*—Fifteen scute males and eleven normal males were obtained from a normal pair. The female offspring were all normal (number not recorded). It is almost certain that the mother was heterozygous in this case, and that the mutation occurred in a previous generation or else very early in her own ontogeny.

### 2. *Scute-2* ($sc_2$)

*Description.*—Scute-2, an allelomorph of scute and scute-3, involves the same scutellar bristles as scute, but varies toward a more extreme condition than this allelomorph. The two anterior scutellar bristles are always missing, frequently one of the posterior scutellars is gone, and occasionally all four are lacking. The bristles on the scutellum are fine and small. In a stock homozygous for reduced and scute-2, both characters are more extreme than either is alone (Fig. 7). Such flies often entirely lack dorso-central and scutellar bristles, and lack one or more orbital bristles.

The compound scute scute-2 females are either somewhat intermediate between the two, or they may look entirely like one or the other component. On the whole, they are more apt to resemble scute-2 than scute.

*Origin.*—From a normal female mated to a scute male, the following types of offspring were obtained: males — one half normal, one half scute-2; females — one half normal, one half somewhat intermediate between scute and scute-2. From this it was concluded that the parent female was heterozygous for the new character and that this character was allelomorphic to scute, a conclusion subsequently verified by direct tests. Six sisters of this female were also tested and none gave scute-2.

### 3. *Scute-3* ($sc_3$)

*Description.*—Scute-3 is an allelomorph of scute and scute-2. All four scutellar bristles, the two sterno-pleural bristles, and a varying number of head bristles are absent. On the head, all three pairs of orbitals are usually missing, and occasionally some of the others are gone.

The compound females involving scute-3 and scute-2 are more apt to be like scute-3 than like scute-2, although in general they are intermediate. Such females could be distinguished from the homozygous scute-2 females in all the cases observed by the absence of at least one sterno-pleural bristle and generally by the absence of all scutellar bristles. Scute-3 males are sterile.

Scute-3 strongly resembles the scute of *melanogaster*. In both cases scutellar and head bristles are affected. Two stocks of *melanogaster* scute kindly examined by Dr. Sturtevant agree with scute-3 in lacking scutellar bristles and orbitals, and in having small ocellar bristles. They both possess post-orbitals, however, and one stock occasionally shows the middle orbital present. The other usually lacks the postverticals.

*Origin.*—Scute-3 was first observed in the offspring of an $F_2$ female from a cross of a scute female from stock by two rough rimmed stump males. This female seemed to

be heterozygous for the new factor, and it was found that the character was also present in males in sister cultures which had been used for stocks.

### *Yellow* (*y*)

*Description.*—In "yellow" flies the body, wings and legs are deep yellow. The bristles and hairs are all yellowish or bronze instead of black. In the latter respect yellow differs from the yellow in *Drosophila virilis* which has black or dark brown bristles and hairs.

*Origin.*—A single yellow male appeared in a bottle of scute rough stump stock.

Yellow was found after the main part of this paper was prepared for publication, and since the experiments involving it have not added materially to the data given in the tables they are omitted from the latter and are given briefly here.

The original yellow scute rough stump male was mated to normal females giving a normal $F_1$. The latter, inbred in pairs, gave 1354 normal daughters and the following classes of sons: normal 488; yellow scute rough stump 466 (non-crossovers 954); yellow scute 25, rough stump 23 (single crossovers in region two 48); yellow scute rough 49, stump 46 (single crossovers in region three 95); yellow scute stump 3, rough 2 (double crossovers involving regions two and three, 5). In addition, two yellow rough stump males and one yellow stump male were obtained. Of the former, one proved to be genetically scute when tested and hence should be in the non-crossover class. The other gave no progeny, but presumably was also a non-crossover. The third fly likewise failed to breed, but since it lacked rough as well as scute it presumably represents a double crossover in regions one and three. It is this fact which leads to the tentative location of yellow above rather than below scute on the map.[4]

[4] This is supported by subsequent data.

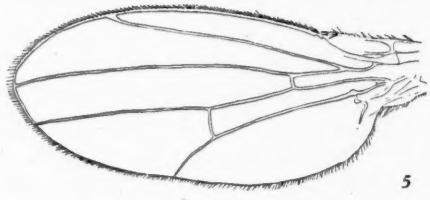
### *Peach* (*p*)

*Description.*—Peach eye color is practically indistinguishable from orange eye color, although, as a rule, it is a trifle darker than orange and does not have the range of shades due to age which are observed in orange. In the same culture, it is impossible to distinguish the two with certainty. The double recessive of peach and orange is probably indistinguishable from either eye color alone. Homozygous peach rough flies have darker eye color than orange rough flies, and are hard to separate from rough alone. Peach eye color is similar to ruby and garnet of *melanogaster* and to rubyoid and carmine of *simulans*.

*Origin.*—A single male with peach eye color was found in a double recessive forked stump stock.

### *Beaded* (*be*)

*Description.*—Beaded refers to the condition of the wings, which have the marginal hairs clumped in irregular patches, especially on the posterior half of the outer margin. The wings are pointed at the ends due to a long notch, extending from the tip of the third vein to about



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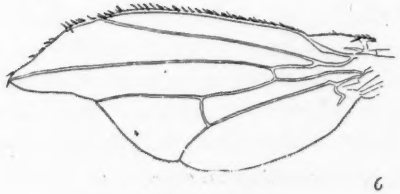

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FIG. 5. Normal, wing.
FIG. 6. Beaded, wing.

the region of the posterior cross-vein, and to the loss of a section from the outside of the wing between the distal ends of the second and third veins (compare Figs. 5 and 6). Beaded flies have poor viability, and the females are sterile. Beaded is similar in appearance to the *melano-*

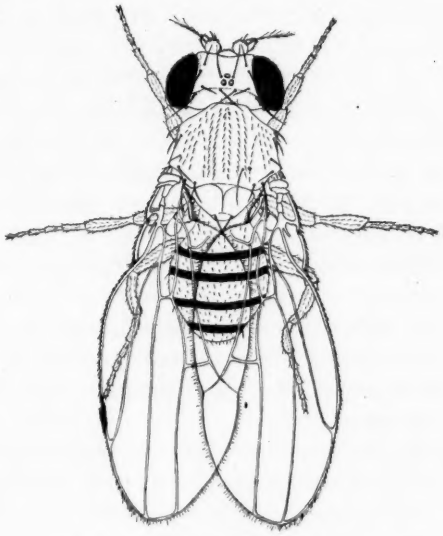


FIG. 7. Reduced scute-2 compound.

*gaster* $cut_6$ although the latter is slightly more extreme than beaded. While $cut_6$ flies are vigorous and fertile, some of the cut allelomorphs are not completely fertile and have poor viability.[5]

*Origin.*—An out-crossed female, known to carry small-bristle rough on one X-chromosome, and small-bristle orange short-3 on the other, produced offspring in which the small-bristle rough males were also beaded. This female was almost certainly heterozygous for the new gene. Nine sisters were bred separately but no beaded flies appeared in their offspring.

[5] Unpublished data for which we wish to thank Drs. Mohr and Bridges.

*Rough (r)*

*Description.*—Rough eye affects mainly the surface of the eye (Fig. 8). When the outer portion of the eye is mounted and examined under the high-power microscope it is seen that the ommatidia are irregular in shape and size with uneven surfaces which are more convex than the normal. The normal eye has regular hexagonal facets with a bristle at every alternate intersection of the sides (See Carnegie Publ. 278, Plate 10, Fig. 3*c* for the normal eye of *D. melanogaster* which has the same arrangement). The bristles of rough eye are irregularly distributed with groups collected in one place and no bristles at all in another. These bristles are about one and a half times the length of the normal ones.

The roughened condition is similar to that found in star eye of *melanogaster* (Carnegie Publ. 278, Text-figure 83). The eyes of *willistoni* rough are also somewhat glossy in texture, and the wing veins are slightly heavier than in the normal flies.

*Origin.*—Several rough males and females were found in one of the bottles of a stock that had been kept in the laboratory for approximately four years. It is probable that the mutant gene had been present in the stock for some time.

*Triple (tr)*

*Description.*—Triple causes four variable wing changes, one or all of which may be present in either or both wings (Fig. 10). (1) The second and third veins may be fused for a short distance at their origin. (2) The wings, slightly tilted up at the ends, are held away from the body at an angle which varies up to about 90°. (3) The third veins fail to reach the distal margin of the wings by amounts which vary from almost nothing to one third the length of the vein. This is particularly evident in the females, where a large section may be missing from the central part of the third vein. (4) An extra cross-vein is present between the second and third

veins at a level about half way between the anterior and posterior cross-veins. This vein, when not wholly formed as a cross-vein, is often indicated by short pieces of disconnected vein.

The fusion of the second and third veins and the extension of at least one wing are constant characters as far as observed. The latter forms the easiest basis of distinguishing this mutant. The females are more extreme than the males in all four of the changes involved. Triple suggests the *melanogaster* mutant, bifid, by its extended wings, fusion of the veins at the base of the wing, and the shortening of one of the veins, although the short vein is the fourth in bifid and the third in triple and the third vein of bifid is thickened at the distal extremity.

*Origin.*—Triple was first noticed in the offspring of a female out-crossed from wild stock. Half the males were triple. On investigation it was found that several bottles of wild stock contained similar males.

Triple males were found six months later in stump stock. Crossed to the original triple stock, these males produced triple female offspring in the $F_1$ generation. The possibility of contamination of the stump stock can be eliminated since the triple males found in the stock were also stump, and there were no cultures containing triple stump flies in the laboratory.

## The Deformed Allelomorphic Series

### 1. *Deformed* (*d*)

*Description.*—Deformed, which involves many parts of the body (Fig. 9), shows sexual dimorphism. In the male the eye is about two thirds the normal size and very rough; in the female the eye is normal in size and only slightly roughened. In both sexes the bristles are abnormally long and irregularly bent. The thoracic hairs are badly disarranged in both sexes, but the effect is very much more exaggerated in the female than in the

male. In the former, the hairs are often clumped in a compact mass on the anterior half of the thorax; while in the male, the hairs are irregularly scattered over the

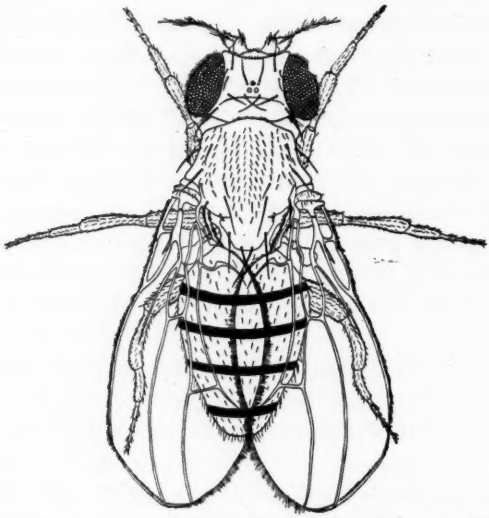


FIG. 8. Rough (eye), rimmed (wing margin), stump (second wing vein) compound.

whole area. The scutellum in the male (sometimes in the female also) is blunt instead of pointed, posteriorly, and the under portions of the thorax are consequently visible. The wings are extended at an angle of about 45° to 90° in both sexes, and the veins are often faint, short, and irregular, especially in the male.

These flies are rather feeble and breed poorly except in mass culture, probably on account of the many physical defects present.

*Origin.*—Many males and females were found in a stock culture of orange forked rough. Sister bottles made up at the same time did not produce any deformed flies.

### 2. *Serrate* (*st*)

*Description.*—Serrate is an allelomorph of deformed, but involves only a part of the characters modified by deformed. The changes in the eyes of the two sexes are exactly the same as those caused by deformed. On the other hand, the bristles and the shape of the scutellum are almost normal and the thoracic hairs are only slightly disarranged. The wings may occasionally be held at an angle with the body, but the venation is practically normal. The only strikingly noticeable effect of serrate is the change in the eyes.

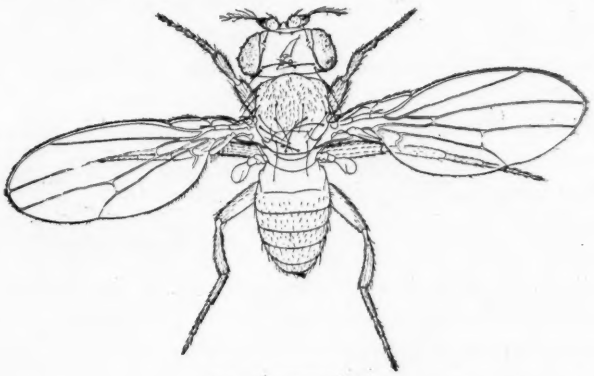


FIG. 9. Deformed ♂.

The compound deformed-serrate females are intermediate between the two allelomorphs but tend to resemble serrate more closely than deformed. Serrate flies are more viable than deformed and breed more readily.

*Origin.*—A single male was found in an $F_2$ mass culture from a mating of two females by one male from scute stock. No other serrate flies appeared in this culture or in a sister culture.

### *Rimmed* (*ri*)

*Description.*—In rimmed flies, a heavy rim of marginal hairs surrounds each wing and the wings curve down

over the abdomen as if the margins were constricted (Fig. 8). The depression between the scutellum and thorax of normal flies is eradicated, leaving a smooth surface at the junction. The thick marginal rim of hairs is the most constant of the effects of rimmed, but the other changes are usually apparent.

*Origin.*—Several males were found in wild type stock.

*Pale (pa)*

*Description.*—The post-vertical and all the thoracic bristles are pale yellow. Occasionally, a few other head bristles are also yellow. The bristles are thin, and the entire fly is weak and small with the wings often not unfolded. None of the original pale flies could be induced to breed. The heterozygous females produced a few pale offspring, but the stock was soon lost.

*Origin.*—The mother of the culture in which pale appeared was heterozygous for scute rimmed on one X-chromosome and for pale morula on the other. Five sisters of this female were bred, but no pale offspring were obtained from any of them. It is impossible to tell whether the mutation to pale occurred in the mother of the culture in which pale was found or whether it took place in her mother.

*Stump (s)*

*Description.*—The distal portion of the second vein is lacking, leaving only a stump at the base of the wing (Fig. 12). This stump varies in length from one quarter to two thirds that of the normal vein.

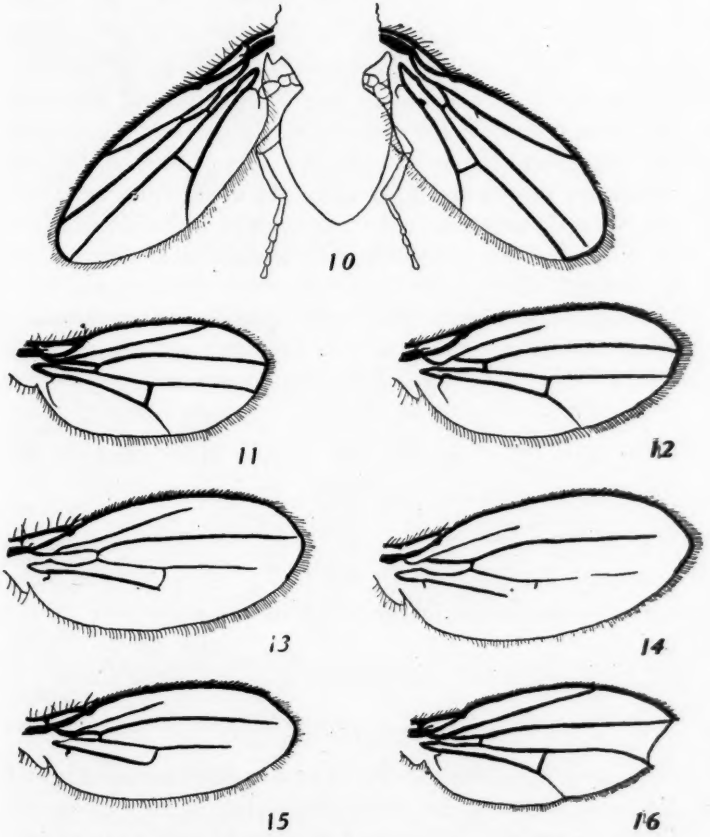
*Origin.*—Six stump males were obtained from a mass culture in which the mothers were heterozygous for forked and the fathers were normal. Four of the stump males were also forked, the others were not.

THE SHORT ALLELOMORPHIC SERIES

1. *Short (sh)*

*Description.*—Typically, all the veins of the wing fail to reach the margin in short flies, although

sometimes the third vein is entire (Fig. 13). The second vein is shorter than any of the others. Ordinarily, the distance between the ends of the veins and the margin is not great. The posterior cross-vein is broken occasionally.




FIGS. 10–16: Fig. 10, triple. Fig. 11, square. Fig. 12, stump. Fig. 13, short ♂. Fig. 14, short-2 ♂. Fig. 15, short-3 ♂. Fig. 16, nicked.

*Origin.*—Short was first observed in half the sons of a single female indicating that the mother was heterozygous for the short gene. This female carried orange

rough on one X-chromosome and stump on the other. The mutation to short evidently affected a locus of the orange rough chromosome not far from the stump locus. Several sister cultures were examined, but no short flies were found.

2. *Short-2* ($sh_2$)

*Description.*—Short-2 is the most extreme of the series. In the females the second, fourth, and fifth veins are very short, the fourth and fifth often not reaching as far as the posterior cross-vein. In cases in which they extend beyond the cross-vein, this vein is broken. In the males the fourth and fifth veins are about three quarters the normal length, and the posterior cross-vein is broken (Fig. 14). The males are indistinguishable from those of short-3.

*Origin.*—A single male was found in small-bristle rough stock.

3. *Short-3* ($sh_3$)

*Description.*—Short-3 is about the same in both sexes (Fig. 15). The second vein is very short, and all the others are about three quarters the normal length. The posterior cross-vein is usually broken.

Females containing any two of these three allelomorphs are intermediate between the two used, with perhaps a closer resemblance to the more extreme member of the pair.

*Origin.*—Several males were found in scute stock.

*Morula* (*m*)

*Description.*—Morula involves a partial roughening of the eyes which is due to a consolidation of a group of facets, especially in the central area of the eye, suggesting the lozenge of *melanogaster*. The viability of this stock is very poor, and the double recessives of morula and any other mutant character rarely survive.

*Origin.*—At least five males were obtained from a mass culture of three pairs which carried rough. The classi-

fication of rough and morula was not accurate at its first appearance.

*Nicked* (*nk*)

*Description.*—Nicked is characterized by irregular notches in one or both wings (Fig. 16). The indentations vary in size and location, but tend to show on the posterior and inner portions of the wing. In certain lines, the character shows regularly, while in certain other lines it overlaps normal a great deal. Flies in which nicked is combined with other mutant characters have rather poor viability.

*Origin.*—Several individuals of both sexes were found in a mass culture.

*Rosette* (*ro*)

*Description.*—In this mutant race, a large number of characters are affected (Fig. 17). The eyes are slightly roughened due to disarrangement of the facets; the thoracic hairs are disarranged, looking as if they had been brushed in the wrong direction; the bristles may be bent; the distal tarsi of the legs may be twisted; and the wings are generally held at an angle with the body, and one or both may be small and circular. The rough eyes and disarranged hairs are constant characters. Rosette flies have very low viability and are hard to breed, especially when other mutant characters are present also.

*Origin.*—Four rosette rough males were obtained among a large number of offspring from one morula male by three rough females.

CONSTRUCTION OF THE X-CHROMOSOME "MAP"

With one or two exceptions the usual procedure[2] has been followed in constructing the chromosome "map." The order of the genes was determined by means of crosses involving three or more loci (Tables III–VI), and that order adopted which, in the consideration of any three points, made the double crossover class the small-

[2] See footnote, p. 213.

est. In most cases the decision was confirmed by several subsequent experiments made for other purposes. Tiny and square have not been definitely located with reference to forked since they proved unsuitable characters for use in linkage experiments. Similarly, the loci of triple and deformed are known to be between rough and rimmed, but the relative positions of the two could not be determined on account of the impossibility of using the two characters together. Pale, morula, and rosette are also only tentatively placed.

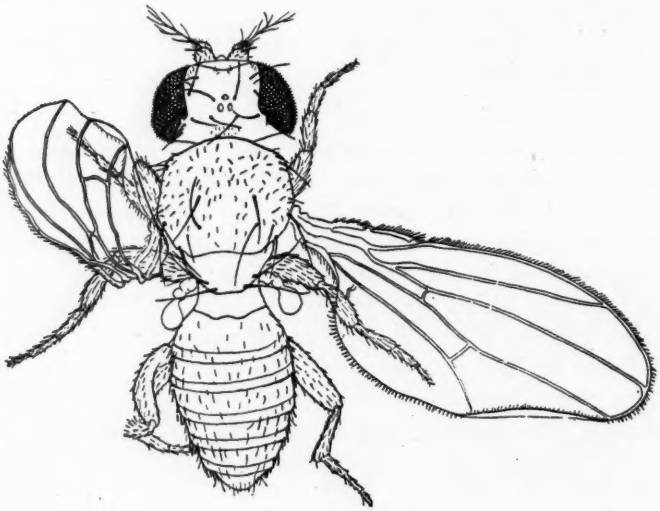


FIG. 17. Rosette.

With the order of the genes established, the "distances," or crossover values, between them were obtained by combining the data from all the experiments given in Tables II–VI. Table VII gives the summary of all data between any two loci in the "map" from those experiments in which no intermediate genes were concerned. This differs from the usual method of summarizing the data in that it includes only experiments in which no intermediate point was used.

As far as possible the positions of the genes on the "map" have been determined by summation of the "distances" between neighboring loci taken in pairs, using stubby arbitrarily as the zero point. In several cases, however, the locus of a gene has been assigned by reference to some main well-established point; notably, orange, forked, scute, rough, or stump. In Table VII, the starred data are those on which the "map" is based. No correction for data involving non-adjacent loci has been made, since the degree of numerical accuracy does not warrant such a computation in this case. No correction has been made for double crossing over in long regions in which no mutant loci are known.

Owing to the possible parallelism between the yellow and scute in *willistoni* and the yellow and scute in *melanogaster* a second set of readings has been given on the "map" using the position of yellow as the zero point and plotting the others in opposite ( + and — ) directions from this. Comparison with the X-chromosome map of *melanogaster* is thus facilitated.

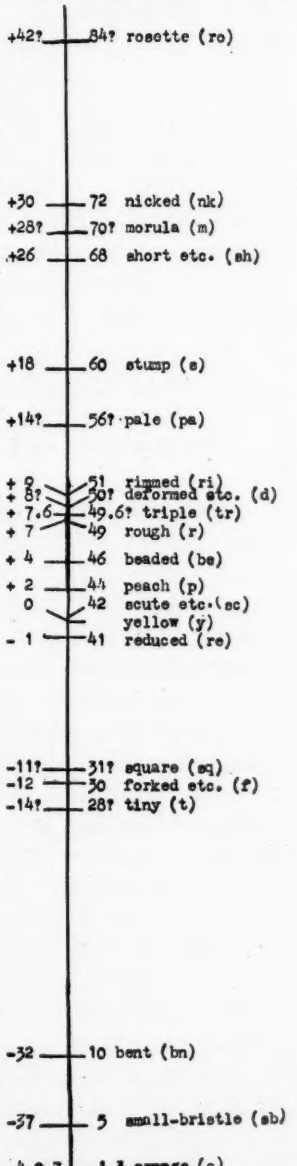



FIG. 18. Map showing linkage relations of sex-linked genes in *Drosophila willistoni*.

## DISCUSSION

The previous work on the comparative genetical study of different species of Drosophila has been concerned largely with species having different types of chromosome groups. It has involved mainly the species *melanogaster, virilis, funebris, simulans* and *obscura.* Of these, only *melanogaster* and *simulans* have the type of chromosome group with which we are concerned here. The published data on the first four of these species have recently been summarized by Sturtevant (1920) and may be passed over briefly. The data on *obscura* are in press and our references to them are made with the kind permission of Mr. D. E. Lancefield.

In *melanogaster, virilis, funebris* and *obscura,* the evidence suggests a tendency on the part of each species to give mutants paralleling those in the others, although the extent of this tendency can not be ascertained accurately because of the impossibility of proving the homology of similar characters. In the case of *melanogaster* and *simulans* the parallelism extends to nearly all of the known *simulans* characters and certain homologies are established by means of hybridization (Sturtevant, '20, '21*a*, 21*b*). To be sure, the two latter species are almost identical and would be expected to give similar genetical results; but it is of interest to note that there is a close resemblance between the proven cases of parallel characters in these, and the apparent cases of parallel characters in the other species. This tends to increase the probability of actual parallelism in the latter where a series of linked characters is involved.

Upon comparing the mutant characters of *willistoni* with those of the others it is evident that only a few striking cases of resemblance are found. Of these the most significant involve the characters yellow and scute. Their morphological resemblances to the yellow and scute in *melanogaster* have already been noted in the

descriptive section. But the evidence for their being parallels is made particularly strong by the fact that their genes are completely or almost completely linked in both species. In *melanogaster* yellow and scute are located at the extreme end (zero point) of the chromosome map, while in *willistoni* they are approximately in the middle.

A situation similar to this has already been found in *D. obscura* (according to unpublished data of D. E. Lancefield). Here the characters yellow and scute also bear a close resemblance to those in *melanogaster* and are very closely linked. As in *willistoni,* the factors for yellow and scute are near the middle of the chromosome map. It will be recalled that *obscura,* like *willistoni,* has a large V-shaped X-chromosome—although the other chromosomes are different (Metz, 1916). In the two species having V-shaped X-chromosomes, then, yellow and scute are "located" near the middle of the chromosome map, while in *melanogaster* with its short, rod-like X-chromosome, yellow and scute are at one end. As Lancefield has pointed out in his discussion of *obscura,* this suggests that one end of the rod-like X in the one case corresponds to the middle of the V-shaped X in the other. And this suggests that the rod-like chromosome itself may correspond to one arm of the V.

The only evidence in *willistoni* on the latter hypothesis is that furnished by the characters forked and stubby. These are possible parallels of the singed and forked in *melanogaster.* They are similar in a general way in the two species (see description above), and the serial order of the genes is the same (Fig. 18), although the linkage relations do not agree exactly.

In this connection it may be recalled that "yellow," "singed" and "forked" have also been found in *Drosophila virilis* (Metz, 1918, and unpublished data), and may, likewise, be considered as possible parallels to those in *melanogaster. Virilis* has a rod-like X-chromosome resembling that of *melanogaster;* and the relative posi-

tions of the three genes on the chromosome map resemble those in *melanogaster*. Yellow is about three units from the end instead of at the end; singed is at about 35 instead of 21 and forked is at about 58 instead of 56.5.

The evidence is not sufficient to warrant the conclusion that these are actually homologous series, but the fact that such series exist suggests that by the present means it may eventually be possible to obtain reliable data for a comparison of the chromosomal make-up of the different species.

Among the other characters in *willistoni* which show some resemblance to characters in *melanogaster* or *simulans* the following may be noted as a matter of record, although there is little indication of their being actual parallels: orange and peach (which look alike) resemble coral or ruby; beaded is similar to the cut allelomorphs both morphologically and in respect to its sterile females and poorly viable males; triple suggests bifid, and morula looks like lozenge. The small bristle of *willistoni* may be comparable to the tiny bristle-2 of *simulans*.

The fact that the X-chromosomes in *willistoni* are morphologically like the large autosomes and not like the X-chromosomes of *melanogaster* suggests that we ought to compare, not only the sex-linked groups of the two species, but also the sex-linked group of *willistoni* with the non-sex-linked groups of *melanogaster*. This has been done, but without revealing any significant indication of parallelism.

In conclusion it may be noted that although the evidence is not yet clear on the genetic relationship of the sex chromosomes in *melanogaster* and *willistoni*, yet if the above suggestion is correct, that the X-chromosome of *melanogaster* corresponds to part of the X-chromosome in *willistoni*, then the resemblance between the chromosome groups of the two species is only superficial. It may also be noted that the genetic "map" of

the X-chromosome of *willistoni* at present is only slightly longer than the map of the *melanogaster* X-chromosome (84 as contrasted with 70 units), whereas the *willistoni* X-chromosome itself appears to be about twice the length of that of *melanogaster*. This suggests that crossing over is less frequent in *willistoni* than in *melanogaster*.

## Summary

1. Twenty-eight recessive sex-linked mutant characters in *Drosophila willistoni* are described and their linkage relations considered.

2. In general, the genetic behavior of *willistoni* (as regards crossing over, etc.) is similar to that of *D. melanogaster* and the other species of Drosophila whose genetic behavior is known.

3. There is a strong indication of parallelism between the mutants yellow and scute in *willistoni* and yellow and scute in *melanogaster*.

4. In both species these characters are completely or very closely linked.

5. There is some indication of parallelism between the characters forked and stubby in *willistoni* and singed and forked in *melanogaster*.

6. In *melanogaster* the genes for yellow and scute are "located" at one end of the chromosome map, and singed and forked are 21 units and 56.5 units respectively from this end. In *willistoni* yellow and scute are near the middle of the map, and forked and stubby are on one side at 12 units and 42 units respectively.

7. Since the X-chromosome of *melanogaster* is short and rod-like, while that of *willistoni* is approximately twice as long and is V-shaped, this relation of the chromosome maps suggests that the *melanogaster* X-chromosome corresponds to one arm of the V-shaped X-chromosome of *willistoni*, with the locus of yellow corresponding in the two cases. This agrees with the suggestion made by Lancefield in the case of *D. obscura* in which the X-chromosomes resemble those of *willistoni*.

8. The comparative lengths of the X-chromosome maps in *melanogaster* and *willistoni* suggests that there is less crossing over in the latter than in the former.

TABLE I

ORIGIN OF MUTANTS

*Explanation of "records":* W indicates R. C. Lancefield records except numbers 1–100 which indicate D. E. Lancefield; L indicates C. W. Metz; R indicates Ruth Ferry.

| Mutant | Symbol | First Found | Record | Parts Affected |
|---|---|---|---|---|
| 1. Stubby | sy | March, 1920 | W 1128 | Bristles. |
| 2. Orange | o | April, 1919 | L 37 | Eye color. |
| 3. Small-bristle | sb | July, 1919<br>July, 1920 | L 336<br>W 1745 | Bristles. |
| 4. Bent | bn | Nov., 1920 | W 1687 | Wings. |
| 5. Forked | f | March, 1919 | L 16 | Bristles; fertility of females. |
| 6. Forked—2 | $f_2$ | March, 1920 | W 1177 | Bristles, hairs; fertility of ♀♀. |
| 7. Tiny | t | Jan., 1920 | W 856 | Bristles. |
| 8. Square | sq | Feb., 1920 | W 965 | Wings; fertility of ♀♀. |
| 9. Reduced | re | Oct., 1919 | W 288 | Bristles; abdomen. |
| 10. Scute | sc | May, 1919 | L 231 | Bristles. |
| 11. Scute—2 | $sc_2$ | Feb., 1920 | W 945 | Bristles. |
| 12. Scute—3 | $sc_3$ | May, 1920 | W 1346 | Bristles; fertility of ♂♂. |
| 13. Yellow | y | Oct., 1921 | R 2 | Color of body, wings, etc. |
| 14. Peach | p | May, 1920 | W 1384 | Eye color. |
| 15. Beaded | be | Nov., 1920 | W 1964 | Wings; viability. |
| 16. Rough | r | March, 1919 | L 9 | Texture of eye. |
| 17. Triple | tr | Dec., 1919<br>May, 1920 | W 754<br>W 1498 | Wings. |
| 18. Deformed | d | Nov., 1919 | W 360 | Almost every part of body. |
| 19. Serrate | st | March, 1920 | W 1146 | Texture and size of eyes. |
| 20. Rimmed | ri | May, 1919 | W 1 | Wings and scutellum; viability. |
| 21. Pale | pa | Feb., 1920 | W 980 | Bristles; viability. |
| 22. Stump | s | June, 1919 | L 254 | Wing vein. |
| 23. Short | sh | Feb., 1920 | W 1110 | Wing veins. |
| 24. Short—2 | $sh_2$ | May, 1920 | W 1440 | Wing veins. |
| 25. Short—3 | $sh_3$ | March, 1920 | W 1164 | Wing veins. |
| 26. Morula | m | | L 411 | Texture of eyes; viability. |
| 27. Nicked | nk | June, 1919 | W 11 | Wings. |
| 28. Rosette | ro | Nov., 1919 | L 438 | Almost every part of body. |

In Tables II–VI parentheses indicate data omitted from the regional summary on account of poor viability of one class or else inability to classify one class. The two columns under the respective headings represent complementary classes, the one to the left that includ-

ing the normal allelomorph of the gene farthest to the left. *E.g.*, in Table II, experiment 3, under crossovers in region 1 there are 48 normal bristle rough eye flies, and 55 stubby bristle normal eye flies.

The plus sign (+) indicates wild-type or normal.

TABLE II

TWO-POINT CROSSES

| Experiment Number | Nature of Cross | Non-crossovers | | Crossovers in Region 1 | | Total |
|---|---|---|---|---|---|---|
| 3 | sy r × + | 71 | 73 | 48 | 55 | 247 |
| 4 | o sb × + | 1,031 | 796 | 34 | 28 | 1,886 |
| 5 | o bn × + | 285 | 275 | 36 | 25 | 621 |
| 20 | f × ri | 66 | 70 | 26 | 20 | 182 |
| 29 | sc × ri | 304 | 291 | 31 | 29 | 655 |
| 35 | p r × + | 131 | 81 | 5 | 7 | 224 |
| 36 | p s × + | 100 | 111 | 20 | 21 | 252 |
| 38 | r ri × + | 127 | 73 | 1 | 1 | 202 |
| 39 | r × s | 173 | 170 | 29 | 26 | 398 |
| 40 | s $sh_3$ × + | 495 | (560) | 37 | (0) | 532 |
| 41 | s × sh | 126 | (169) | 14 | (0) | 140 |

TABLE III

THREE-POINT CROSSES

| Experiment Number | Nature of Cross | Non-crossovers | | Crossovers in Region 1 | | 2 | | 1, 2 | | Total |
|---|---|---|---|---|---|---|---|---|---|---|
| 2 | sy sb × bn | 704 | 582 | 28 | 29 | 40 | 38 | 0 | 0 | 1421 |
| 6 | o bn × $f_2$ | 35 | 66 | 6 | 8 | 16 | 8 | 3 | 0 | 142 |
| 18 | f r × re | 243 | 238 | 40 | 35 | 12 | 25 | 0 | 0 | 593 |
| 19 | f s × sc | 48 | 50 | 10 | 9 | 14 | 14 | 1 | 0 | 146 |
| 23 | sc ri × r | 230 | 189 | 20 | 17 | 3 | 3 | 0 | 0 | 462 |
| 26 | sc r s × + | 510 | 460 | 26 | 28 | 55 | 45 | 4 | 2 | 1130 |
| 28 | sc s × d | 258 | 324 | 23 | 25 | 25 | 34 | 1 | 1 | 691 |
| 32 | sc ri × m | 198 | 265 | 42 | 28 | 60 | 26 | 0 | 6 | 625 |
| 33 | sc s $sh_3$ × + | 174 | (146) | (54) | 55 | (8) | 19 | 3 | (0) | 251 |
| 34 | p r × be | (20) | 168 | 3 | (0) | (1) | 5 | 0 | 0 | 176 |
| 37 | r ri × tr | 312 | 281 | 2 | 0 | 1 | 5 | 0 | 0 | 601 |

TABLE IV

FOUR-POINT CROSSES

| Experiment Number | Nature of Cross | Non-crossovers | | Crossovers in Region | | | | | | | | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 1 | | 2 | | 3 | | 1, 2 | | 1, 3 | | 2, 3 | | 1, 2, 3 | | |
| 1. | sy r × o sb | 321 | 325 | 9 | 4 | 22 | 12 | 244 | 205 | 0 | 0 | 1 | 1 | 7 | 4 | 0 | 0 | 1,156 |
| 7. | o re × f s | 40 | 50 | 15 | 20 | 3 | 13 | 15 | 13 | 1 | 2 | 6 | 5 | 1 | 3 | 0 | 0 | 187 |
| 9. | o f r × ri | 161 | 150 | 70 | 88 | 58 | 51 | 10 | 2 | 8 | 10 | 2 | 4 | 2 | 1 | 0 | 1 | 618 |
| 10. | o r s × t | 48 | 57 | 30 | 27 | 15 | 23 | 7 | 15 | 5 | 1 | 3 | 3 | 1 | 1 | 2 | 2 | 240 |
| 12. | o r s × tr | 37 | 56 | 40 | 50 | 0 | 1 | 6 | 7 | 0 | 0 | 8 | 2 | 0 | 0 | 0 | 1? | 208 |
| 13. | o d × r ri | 12 | 19 | 6 | 7 | 0 | 2 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 48 |
| 15. | f re $sc_2$ × r | 290 | 311 | 54 | 43 | 9 | 4 | 43 | 26 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 781 |
| 21. | re $sc_2$ × be r | (89) | 268 | 1 | (0) | 4 | (0) | (3) | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 279 |
| 22. | sc r s × d | 220 | 160 | 16 | 20 | 8 | 0 | 14 | 13 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 455 |
| 24. | sc ri × r s | 348 | 306 | 41 | 18 | 6 | 12 | 25 | 17 | 0 | 1 | 3 | 2 | 0 | 0 | 0 | 0 | 779 |
| 27. | sc r s × nk | 250 | 306 | 31 | 23 | 42 | 27 | 28 | 10 | 1 | 4 | 3 | 9 | 1 | 19 | 0 | 0 | 754 |
| 30. | sc ri × pa m | (38) | 108 | 10 | (2) | 7 | (0) | (6) | 7 | 0 | 0 | 0 | (1) | 0 | 0 | 0 | 0 | 132 |
| 31. | sc ri m × s | 149 | 43 | 2 | 15 | 12 | 6 | 12 | 13 | 2 | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 257 |

TABLE V

FIVE-POINT CROSSES

| Experiment Number | Nature of Cross | Non-crossovers | | Crossovers in Region | | | | | | | | | | | | | | | | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 1 | | 2 | | 3 | | 4 | | 1, 2 | | 1, 3 | | 1, 4 | | 2, 3 | | 2, 4 | | 3, 4 | | 1, 3, 4 | | |
| 8... | o f × $sc_3$ ri s | 64 | 58 | 27 | 31 | 9 | 9 | 11 | 2 | 6 | 2 | 2 | 2 | 1 | 3 | 3 | 2 | 1 | 0 | 1 | 2 | 1 | 2 | 0 | 0 | 239 |
| 16... | f re × sc r s | 167 | 204 | 20 | 18 | 3 | 3 | 8 | 6 | 17 | 28 | 0 | 0 | 0 | 1 | 2 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 480 |
| 17... | f re $sc_2$ r × ro | 232 | 222 | 41 | 27 | 1 | 1 | 21 | 14 | 190 | 75 | 0 | 0 | 0 | 0 | 6 | 21 | 0 | 0 | 1 | 0 | 2 | 11 | 1 | 0 | 866 |
| 25... | sc ri × r s nk | 122 | 140 | 10 | 7 | 2 | 0 | 11 | 0 | 27 | 6 | 0 | 0 | 0 | 2 | 0 | 5 | 0 | 0 | 0 | 3 | 0 | 14 | 0 | 1 | 350 |

TABLE VI

SIX-POINT CROSSES

| Experiment Number | Nature of Cross | Non-crossovers | | Crossovers in Region | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 1 | | 2 | | 3 | | 4 | | 5 | | 1,2 | | 1,3 | | 1,4 | | 1,5 | | 2,4 | | 2,5 | | 3,4 | | 3,5 | | 4,5 | | 1, 2, 3 | | 1, 2, 5 | |
| 11. | o sq r s × sc ri | 92 | 60 | 41 | 50 | 19 | 4 | 10 | 4 | 2 | 1 | 9 | 9 | 3 | 3 | 2 | 3 | 1 | 2 | 1 | 6 | 0 | 1 | 1 | 0 | 1 | 2 | 0 | 0 | 0 | 1 | 0 | 2 | 331 |
| 14. | f re $sc_2$ r × p s | 180 | 152 | 16 | 18 | 1 | 1 | 3 | 3 | 11 | 8 | 22 | 18 | 0 | 0 | 0 | 0 | 0 | 1 | 2 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 1 | 0 | 0 | 0 | 442 |

TABLE VII

SUMMARY OF ALL AVAILABLE DATA BETWEEN CONSECUTIVE LOCI

| Region | Cross-over Value | Number of Flies | Region | Cross-over Value | Number of Flies | Region | Cross-over Value | Number of Flies |
|---|---|---|---|---|---|---|---|---|
| sy–o[6] | 1.3 | 1,156 | t–r[6] | 20.8 | 240 | r–a[6] | 2.4 | 503 |
| sy–sb | 4.0 | 1,421 | sq–sc[6] | 10.6 | 331 | r–ri[6] | 2.3 | 2,742 |
| sy–r | 41.7 | 247 | re–sc[6] | 0.95 | 2,848 | r–s[6] | 11.1 | 3,444 |
| o–sb[6] | 3.5 | 3,042 | re–r | 6.2 | 593 | r–ro[6] | 35.4 | 866 |
| o–bn | 10.2 | 763 | re–s | 23.0 | 187 | tr–ri[6] | 1.0 | 601 |
| o–f[6] | 29.0 | 1,044 | $sc_2$–p[6] | 1.8 | 442 | tr–s | 11.5 | 208 |
| o–t | 30.4 | 240 | $sc_2$–be | 1.4 | 279 | d–ri[6] | 4.2 | 48 |
| o–sq | 34.7 | 331 | sc–r[6] | 7.1 | 6,388 | d–s | 7.9 | 1,146 |
| o–r | 44.5 | 256 | sc–d[6] | 7.2 | 691 | ri–pa[6] | 5.3 | 132 |
| sb–bn[6] | 5.5 | 1,421 | sc–ri | 9.9 | 1,908 | ri–s | 7.5 | 1,956 |
| sb–r | 40.0 | 1,156 | sc–s | 21.9 | 397 | ri–m | 14.7 | 625 |
| bn–$f_2$ | 19.0 | 142 | p–be[6] | 1.7 | 176 | pa–m | 5.3 | 132 |
| f–re[6] | 11.2 | 3,349 | p–r | 5.0 | 654 | s–sh[6] | 7.9 | 923 |
| f–sc[6] | 11.9 | 385 | p–s | 16.3 | 252 | s–m[6] | 10.1 | 257 |
| f–r | 21.2 | 618 | be–r | 2.4 | 455 | s–nk[6] | 11.5 | 1,100 |
| f–ri | 25.3 | 182 | r–tr[6] | 0.5 | 809 | | | |

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[6] These data were used in the construction of the chromosome "map."

# INHERITANCE OF PLUMAGE COLOR IN CROSSES OF BUFF AND COLUMBIAN FOWLS

DR. L. C. DUNN[1]

As a part of a search for material suitable for use in measuring the linkage strength of several sex-linked characters in poultry, some preliminary experiments have been undertaken on the inheritance of the Columbian plumage pattern. The results of these experiments have confirmed those of Sturtevant (1912) in establishing the sex-linked nature of one of the genes involved in the production of this pattern, and have demonstrated the relationship between it and the buff plumage coloration. The inheritance and somatic effects of the chief factor involved appear to be clear enough to make it useful in genetic investigations on poultry. A short description of the experimental results is therefore given here, to be followed by a more detailed report when further evidence is at hand.

The Columbian pattern, sometimes known as the Ermine coloration, is characteristic of several standard varieties of a number of breeds of poultry of which the Light Brahma, the Columbian Plymouth Rock and the Columbian Wyandotte are perhaps the most familiar. Although subject to some variation the pattern consists in general of a pure white surface color in all parts of the plumage except in the hackles, wings, and tail feathers, in which black is present either as a central stripe (hackles); as a solid color covering somewhat more than half the feather (primaries) or as a solid color covering the whole feather (tail). In typical Columbian fowls the undercolor or fluff at the base of the body feathers is generally lead or slate, which is sometimes so pronounced as to show through at the surface especially on the back.

[1] Contributions in *Poultry Genetics*, Storrs Agr. Exp. Station.

This pattern is alike in both sexes except for the slightly different appearance caused by structural differences in the hackle and saddle feathers.

The down color of newly hatched Columbian chicks is white or a yellowish white like the down characteristic of the chicks of many white varieties of poultry, *e.g.*, White Leghorns. Black or gray markings appear on most Columbian chicks as a spot on the head, or as dorsal stripes on the head or back and in the developing wing quills. This pattern varies in different individuals from an entire absence of dark pigment to the presence of rather heavy dark dorsal stripes.

The color variety chosen for crossing with Columbian was buff, since this offered a clear contrast in the absence of white and the uniformity of coloration and because the plumage color of both sexes is practically the same. Moreover, buff is known to be recessive to many other plumage colors and patterns and for this reason is less likely to carry other factors which might complicate the results.

The first crosses were made between a Columbian male extracted from the second generation of a cross between Light Brahma and White Leghorn,[2] and purebred Buff Orpington females.

Twenty-three chicks were hatched from these crosses. Of these, twelve were predominantly white in the down, and eleven were buff. Of the whites four were pure white, five had a black streak or spot on the head and saddle, and black pin feathers appearing in the wings, and closely resembled purebred Light Brahma chicks in color; one was smoky white and two were white with a buff spot or streak on the head and neck. Of the buffs eight were clear buff in color, one was a very light buff

[2] The cross of Light Brahma by White Leghorn (quoted by permission from unpublished data of Sinnott and Warner) when made reciprocally produced white birds in $F_1$, generally with some ticking with black and occasional brassiness or tinging with buff. The White Leghorns used were apparently pure for the dominant inhibitor of color (I) while the Light Brahmas contained the recessive allelomorph of this gene (i).

and two were buff with black down on heads and saddles and with black feathers in the wings. All of the white chicks developed adult plumage resembling the Columbian pattern except that the black in the hackles, tail and wings was a dingy gray, occurring as stippling on the white ground rather than as a solid color. The buff chicks which survived developed adult plumage in which the hackles, tail and wing feathers were gray or black, while the feathers over the rest of the body were buff. These resembled mosaics of buff and Columbian in which the Columbian pattern was imposed on a buff ground.[3]

In tabular form the results of this cross were as follows:

TABLE I

Columbian Male × Buff Female

| | White | | Buff | |
|---|---|---|---|---|
| Down Colors | 12 | | 11 | |
| | ♂ | ♀ | ♂ | ♀ |
| Adult Colors | 6 | 6 | 3 | 6 |

The appearance of two kinds of offspring in equal numbers from this cross indicated that one parent was probably heterozygous in a factor causing the difference between white and buff. Later work showed this to be the male. When a *purebred* Light Brahma male was mated to purebred buff females (Orpingtons and Plymouth Rocks) the thirty-seven offspring were without exception white in the down and developed into white Columbians as adults. The dominance of white over buff was practically complete although one or two buff feathers were noted on one hybrid and a slight buff tinge on another.

[3] The resemblance of these hybrids to descriptions and illustrations of early buff varieties (Tegetmeier, 1872) is quite striking. At present the only buff variety characterized by a considerable amount of black in hackles, wings and tail is the Buff Brahma, although this is not yet recognized by poultrymen as a standard type.

The coloration characteristic of the Rhode Island Red breed is essentially an ermine or Columbian pattern with a red ground substituted for the white of true Columbians. A very useful discussion of the relationships between these patterns from the standpoint of a breeder and fancier of long experience is given by Robinson (1921) see esp. pp. 55 and 56.

The offspring of the purebred Light Brahma male by buff females were much whiter than the chicks from the first male. Only one of the thirty-seven showed the dark head spot characteristic of Light Brahma chicks and all were of a clear dead white, lacking even the yellowish tinge characteristic of most white chicks in the down. As adults these birds were very similar to the first lot. The amount of black in hackles, tails and wings was about intermediate between the amount present in the Columbian parent and the absence of black in pure white birds.

The first generation hybrid chicks were crossed in two ways. The $F_1$ Columbian females were backcrossed to a purebred Buff Plymouth Rock male; the $F_1$ buffs were bred *inter-se*. The results of these matings are presented in Tables II and III.

TABLE II

RESULT OF CROSSING $F_1$ COLUMBIAN FEMALES WITH PURE BUFF MALE

| | White | | Buff | | Total |
|---|---|---|---|---|---|
| Down Colors | 36 | | 38 | | 74 |
| | Columbian | | Buff | | |
| | ♂ | ♀ | ♂ | ♀ | |
| Adult Colors | 14 | 0 | 0 | 21 | 35[4] |

TABLE III

RESULT OF CROSSING $F_1$ BUFFS INTER-SE

| | Buff and White | | Buff | | Total |
|---|---|---|---|---|---|
| Down Colors | 9 | | 74 | | 83 |
| | Columbian | | Buff | | |
| | ♂ | ♀ | ♂ | ♀ | |
| Adult Colors | 0 | 0 | 17 | 15 | 35[4] |

From the backcross of $F_1$ Columbian females with a buff male equal numbers of buff and white chicks resulted, a clear monohybrid segregation. Evidently one factor determines the difference between white and buff, and from the $F_1$ results it is clear that white is the dominant allelomorph. This factor is however sex-linked, since

[4] The differences between the numbers of chicks and the numbers of adults indicate the number of birds which died before definitive plumage or secondary sex characters were developed.

all sons of the $F_1$ Columbian females are white (Columbian) while all the daughters are buff.

The mating of $F_1$ buffs *inter-se* produced only buff chicks, indicating that buff is recessive and breeds true. The nine chicks recorded as buff and white all had buff heads or wings or both and those which lived developed buff adult plumage. Genetically they were probably extremely light buffs.

As regards only the difference between white and buff, we may conclude that the Columbians contain a dominant sex-linked gene for the inhibition or restriction of buff from the plumage. The first male was evidently heterozygous for this factor; the second male was homozygous for it; the Columbian females contained but one dose of it, and this was located in the single sex chromosome; while all the buffs lacked it entirely. This is evidently the same gene (I) which Sturtevant (1912) found in Columbian Wyandottes, although its effects were somewhat obscured by other factors in his crosses with Brown Leghorn.

The presence of this gene in some White Wyandottes which I have studied strengthens the homology between the gene with which Sturtevant was dealing and the gene which is present in the Light Brahmas used in these experiments. I have recently crossed two White Wyandotte males with purebred Buff (Orpington) females. The white males were known to be recessive white (cc), *i.e.*, they lacked the gene (C) for the development of color in the plumage. The results of this cross are shown in the table following.

Result of Crossing White Wyandotte Males with Buff Orpington Females

| | White | | | Buff | | Total |
|---|---|---|---|---|---|---|
| Down Colors | 13 | | | 13 | | 26 |
| | Columbian | | | Buff | | |
| | ♂ | ♀ | ? | ♂ | ♀ | |
| Adult Colors | 3 | 2 | 1 | 3 | 7 | 16 |

In addition to the types noted above three unclassified

chicks were born from one mating. These were chiefly white in the down with black spots on the crown and neck and black quills in the wings. They resembled very dark Columbian chicks. These developed adult plumage different from the other chicks in this cross and could not be classified either as Columbians or buffs. Additional factors which have not been identified were probably contributed by one of the White Wyandotte males and further reference to these birds will therefore be postponed until more information is obtained. Omitting these, the salient fact concerning this cross is the production of only two classes of chicks, white (Columbian) and buff in equal numbers. The White Wyandotte males bred, therefore, like $F_1$ hybrids between Columbian and buff and were undoubtedly heterozygous in the gene for the restriction of buff. As adults the offspring of this cross were indistinguishable in color from the offspring of the heterozygous Light Brahma male first used in crosses with buffs. The amount of black in the hackles of these birds appeared to be somewhat greater than in the offspring of the Light Brahma cross, but it was not sufficient to serve as a distinguishing mark. White Wyandottes, therefore, may carry a gene for the restriction of buff which is probably the same as the gene found in Light Brahmas and Columbian Wyandottes.[5] It is not demonstrable, of course, except in crosses of White Wyandottes with colored fowls which supply the dominant gene *C* for the development of pigment.

In addition to these three instances of the occurrence of a gene for restriction of buff there are numerous other cases in the literature in which the difference between buff (or red) and white in certain parts of the plumage is apparently due to the same gene or one with similar effects. Davenport (1912) found a sex-linked dif-

[5] Professor W. A. Lippincott has called my attention to this statement in Robinson (1921), p. 42: "The pattern (*i.e.*, Columbian) was also produced by crossing the Rhode Island Red (which has really the same pattern with the black—on a red ground—reduced to a minimum) with a White Wyandotte."

ference between Dark Brahmas and Brown Leghorns, the gene "W" inhibiting the appearance of buff or red in the hackles and saddles of Dark Brahmas. Jones (1914) was probably dealing with a similar sex-linked gene in his crosses between Silver and Golden Campines, and Hagedoorn's (1914) evidence indicates that the same or a similar sex-linked gene differentiates Silver and Golden Assendelvers. Punnett (1919) distinguishes a sex-linked gene "S," which in crosses of Silver and Golden Campines inhibited the development of buff or gold in the plumage, leaving certain portions of the feather "silver" or white. Most recently evidence presented by Haldane (1921) indicates that black and white barring such as characterizes the Barred Plymouth Rock variety is differentiated from black and buff (or red or gold) barring by the same gene "S" for the inhibition of buff. This sex-linked gene "S," Haldane found to be linked, as was to be expected, with the sex-linked gene "B" (barring).

In each of these cases a dominant sex-linked gene was found which restricted or inhibited the development of buff (or red or gold) in certain parts of the plumage. Although in the absence of data on crosses between the varieties mentioned it is impossible to assert that the restriction of buff in the silver or white-patterned varieties is in each case due to the same gene, the presumptive evidence in favor of such a view is strong. It appears probable that Columbian and buff varieties of several breeds (Leghorns, Plymouth Rocks, Wyandottes, etc.) are differentiated by the presence in the Columbians of a gene for the inhibition or restriction of buff pigment; and in view of the history of the various color varieties that this gene has been introduced into and now differentiates Golden from Silver-laced Wyandottes, Golden from Silver Spangled Hamburgs, gold pencilled (or partridge) varieties from silver-pencilled ones and other golden varieties from silver varieties which differ only in the distinction between buff and white in the plumage.

Data on the results of crosses involving these color varieties are urgently needed, and the generalization offered above is put forward as a temporary simplification in lieu of but as an aid to more extensive research.

### The Black Component of the Columbian Pattern

When the experiments with buff and Columbian fowls were begun it was supposed that at least two alternative characters distinguished these varieties; viz., ground color (white as opposed to buff) and pattern (black in hackles, wing, and tail as opposed to self coloration). The results of these experiments, a reexamination of the parent types and a cursory review of poultry literature indicate the error of this assumption.

1. *Experimental.*—The first generation of the cross Columbian × Buff consisted of birds intermediate between the parental types in the amount of black pigment present. If four arbitrary grades (1–2–3–4) in the reduction of the amount of black in hackles, wing and tail are made between typical Columbian and entire absence of black (white or buff self) then the first generation is found to consist of the following grades.

| Columbian | —1 | —2 | —3 | —4 | Self |
|---|---|---|---|---|---|
| 0 | 8 | 10 | 2 | 0 | 0 |

If the buff parents are classified as self then the hybrids resemble the Columbian parent more closely. But a careful examination of all the buff females used revealed the presence in each of them of a small amount of black pigment usually as broken patches or fine stippling in the tail and primary feathers, and occasional traces in the hackles.[6] The buffs, therefore, can not be regarded as selfs and most of those used in these experiments were assignable to grade–4. The amount of

[6] It is the experience of farmers and poultrymen, as evidenced in poultry literature, that buff fowls with no admixture of black pigment have been rare. Black in wings and tail is being rigidly selected against and is being gradually reduced in modern breeds.

black in the $F_1$ fowls is only slightly above the mid point between Columbian and grade–4.

An $F_2$ generation has not yet been raised from the cross of typical Columbian × Buff, but some data are available on the $F_2$ generation from the cross of the heterozygous Light Brahma which was first used in crosses with buff. This male was lighter than typical Columbian, about grade–1. His offspring were not graded but had slightly less black than the offspring of the typical Columbian male, averaging about grade–3. The amount of black was similar in the Columbian and buff progeny. When these $F_1$ buffs were bred *inter-se* the grades of the $F_2$ adult fowls were as follows:

| Columbian | —1 | —2 | —3 | —4 | Self |
|---|---|---|---|---|---|
| 0 | 7 | 13 | 10 | 1 | 0 |

The variation in amount of black pigment was practically continuous, except that the Columbian parental type was not recovered. No buffs were obtained which were entirely free from black in tails or wings.

The $F_1$ Columbians were backcrossed with a pure Buff Rock male which showed only faint traces of black stippling (mealiness) in the tail. The progeny of this cross were of the following grades:

Whites (Columbians) and buffs combined:

| Columbian | —1 | —2 | —3 | —4 | Self |
|---|---|---|---|---|---|
| 0 | 2 | 3 | 9 | 13 | 4 |

The amount of black in these fowls was obviously much less than in the $F_2$ generation although the same grades were represented. In four fowls (three buffs and one white) no trace of black pigment could be detected.

It is obvious from these facts that as regards the black component of the Columbian pattern, the Light Brahmas and the buffs used differ only in amount. A blend occurs in the first generation followed by segregation in the second and backcross generation. It is probable, there-

fore, that the two types crossed differ from each other by multiple factors affecting the amount of black pigment produced. The number of these factors can not be estimated because of the small numbers of animals involved and because a second generation has been bred only from an original cross in which the Columbian parent did not have the amount of black normal to that variety. Failure to recover the typical Columbian pattern in later generations is probably due to the last named circumstance rather than the absence of segregation.

The two varieties probably do not differ by any single factor determining the presence or absence of black pigment, but only in the degree to which black is produced, the degree probably being governed by accessory or modifying factors. This fact attaches especial interest to the appearance of several birds in the backcross generation which show no trace of black pigment. Do these represent loss of a factor determining the ability to develop any black pigment at all or are they segregates in which factors limiting the exercise of the black-producing function are at a maximum? Since they are few in number and since variation in the amount of black grades imperceptibly into the self condition I am inclined to the latter view. If this is true it should be possible to reduce the amount of black in Columbian fowls by rigid selection against it to such a point that birds might be produced which were phenotypically white, but which as regards restriction of buff would breed like Columbians.[7] Such a character would be in effect a sex-linked self white and the absence of a sex-linked white in the many breeds investigated points to the probability that none has been produced in this way.

Much of the interest in the case presented here inheres in the apparent simplicity of the results. The crossing of Light Brahmas and Buff Orpingtons or Buff Plymouth Rocks produces in the first, second and backcross generations only two easily distinguishable types, white (Co-

[7] One such bird has appeared in the course of these experiments; see p. 244.

lumbian) fowls and buffs—in which to be sure there is some variation in the amount of black pigment present in certain parts of the body, but apparently no epistatic pattern factors are introduced from either side of the cross to obscure the visible segregation of the main factors. Restriction of buff as found in Columbian fowls is, therefore, a valuable sex-linked gene for use in measuring linkage or in other Mendelian experiments with poultry while buff appears to be the best color variety to be used in studying the inheritance of unknown plumage characters. The Brown Leghorn or game type plumage pattern, although it resembles the supposed wild type form, is in the writer's opinion less valuable than buff because of the often evidenced[8] presence in the genetic constitution of the Brown Leghorn of epistatic pattern factors for extension of black pigment, stippling, etc.

The general results of the experiments reported have been to confirm and extend the previously known facts regarding the inheritance of the Columbian variation. The genetic relationships of this pattern and the buff coloration also throw some interesting light on the evolution of these two color varieties. They differ, it has been shown, only in one main gene which determines the production or restriction of buff in the plumage. Both are able to develop black pigment in certain parts of the plumage, while they differ quantitatively in the degree to which black may be produced. The former single factor difference probably arose as a single mutation, while the latter and less important difference is one which could be brought about by selection of small variations which had already arisen in a common parental stock.

The variation which produced the chief difference between these two color varieties, *i.e.*, the restriction of buff, undoubtedly took place at least 75 years ago and probably in China, although there is no evidence that the same variation has not occurred several times. The first known Columbian breed was probably the Gray Shang-

[8] Sturtevant, A. H. (1912); Lefevre, G. (1916).

hae, from which the Light Brahmas were derived. These fowls were imported into the United States from China in the decade before 1850[9] and into England shortly afterward. At about the same period and often in the same shipments were imported certain buff birds which eventually became the foundation stock of the Buff Cochin breed from which practically all buff varieties of the present day received their color. These two varieties were practically identical in characters other than plumage color[10] and in the matter of plumage the chief difference was the difference in body feathers, being white more or less stippled with gray in the Shanghaes and buff similarly stippled with gray (mealiness) in the Buff Cochins. In China, observers have regarded the buff as the older color variety while the gray was noted as separate about 1840.[11] The Chinese apparently paid little attention to color in breeding their fowls and the variation from buff to white (or the reverse) in the plumage may have occurred many years or even centuries previous to this date.

The further differences between Columbian and Buff breeds have taken place since their introduction from the Orient, chiefly under the selective breeding of English and American poultrymen. The buffs were at first characterized by a great deal of variation in the shade of the principal color—ranging from lemon to red; while the wings, and tails, and tips or margins of the hackles varied from solid black through stippling and blotching to an absence of black in any one of these parts.[12] All subsequent selection has been against the black[13] and the American Standard of Perfection now specifies "buff in all parts of the plumage." In the Shanghaes or Light Brahmas on the other hand the object of the breeder

[9] Weir, Johnson and Brown, "The Poultry Book," N. Y., 1912, p. 528.

[10] Tegetmeier, W. B., *loc. cit.*, p. 63.

[11] Weir, Johnson and Brown, *loc. cit.*, p. 528.

[12] Weir, Johnson and Brown, *loc. cit.*, p. 527; p. 630, p. 540. Tegetmeier, *loc. cit.*, pp. 40–41.

[13] With the exception of the selection for black in hackles, wing and tail which was employed in developing the Buff Brahma variety.

has been to preserve the black in the hackles, wings and tails and to heighten the contrast with the white body by selecting against grayness or mealiness in the body feathers. Two principal processes were apparently involved in the production of buff and Columbian varieties; a discontinuous change or mutation producing the chief difference, and the accumulation by selection of minor factors producing the minor changes. It is impossible to say whether the buff and Columbian varieties which exist at the present time in the principal breeds were derived from these original types by crossing or whether the principal mutation and the minor changes and selection have recurred in the different breeds. The probabilities are in favor of the first alternative.

## SUMMARY

1. The Columbian plumage coloration in domestic fowls is distinguished from buff coloration by the presence of a gene *S* which determines the restriction or inhibition of buff pigments from the feathers. This gene is sex-linked, and dominant over its allelomorph *s*, which permits the development of buff pigment.

2. Fowls with the Columbian coloration do not differ from buff fowls in any single gene governing the development of black pigment. Multiple genes appear to determine the difference in the amount of black pigment developed.

3. Columbian and buff fowls are genetically alike in plumage pattern, that is, in the ability to develop black pigment in the feathers of certain areas (hackle, wing and tail feathers).

4. The buff coloration appears to have diverged from the Columbian coloration, or the reverse, by a single gene mutation affecting the development or inhibition of buff pigment; and by the accumulation through artificial selection of multiple genes for the development of black pigment in the Columbian varieties of fowls, and by the reverse selection in most buff varieties.

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# FURTHER NOTES ON THE PALEONTOLOGY OF ARRESTED EVOLUTION

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THE writer has in a former paper[1] endeavored to follow up the causes of persistence as seen from the side of the paleontologist. Using as a basis the genera which appear in Zittel-Eastman's Textbook of Paleontology (1913) and defining as persistent all genera which pass through more than two periods, the following data relative to number of persistent genera (*A*), total number of genera cited (*B*), and percentage of persistent genera (*C*) were obtained:

| | *A* | *B* | *C* |
|---|---|---|---|
| Foraminifera | 48 | 86 | 56 |
| Sponges | 9 | 149 | 6 |
| Corals | 46 | 237 | 15 |
| Echinodermata: | | | |
| Crinoidea | 5 | 277 | 2 |
| Cystoidea | 0 | 96 | 0 |
| Blastoidea | 1 | 23 | 4 |
| Ophiuroidea | 0 | 25 | 0 |
| Asteroidea | 5 | 43 | 11 |
| Echinoidea | 19 | 191 | 10 |
| Bryozoa | 68 | 306 | 22 |
| Brachiopoda | 33 | 384 | 9 |
| Mollusca: | | | |
| Pelecypoda | 78 | 446 | 16 |
| Scaphopoda | 5 | 18 | 27 |
| Gastropoda | 126 | 420 | 30 |
| Pteropoda (so-called) | 5 | 17 | 29 |
| Pulmonata | 7 | 65 | 11 |
| Cephalopoda (*a*) Nautiloidea | 12 | 170 | 7 |
| (*b*) Ammonoidea | 0 | 455 | 0 |
| (*c*) Dibranchiata | 0 | ... | 0 |
| Crustacea | | | |
| Trilobita | 6 | 131 | 4.5 |
| Ostracoda | 18 | 68 | 26.5 |
| Cirripedia | 4 | 20 | 20 |
| Malacostraca | 7 | 134 | 4.5 |
| Arachnida | 3 | 66 | 4.5 |
| Selachii | 16 | 168 | 9.5 (in first edition) |

[1] R. Ruedemann, "The Paleontology of Arrested Evolution." Presidential Address. Albany, 1916. New York State Museum Bull. 196, 1918, pp. 107–138.

Dipnoi, Teleostei, Reptilia each have one in the 1896 edition of Zittel-Eastman. The vertebrate volume had not yet appeared of the second edition.

From an analysis of the percentages we drew the following inferences:

1. The lower classes tend in general to have more persistent types than the higher.

2. Within each order and class, again, the lower subclasses tend to furnish the greater percentage of persistent forms.

3. Frequently the persistent genera form a primitive central stock from which numerous shorter lived genera branch off.

4. The stable conditions of the open ocean and deep sea (as in the Foraminifera) and the subterranean conditions favor persistence of types, the latter condition including the burying and boring forms.

5. Sessile forms contain more persistent types than the vagile benthos.

6. Persistent types prevail in much greater number among the marine forms than among the land and freshwater animals. Among the continental forms again the limnal and fluviatile forms appear to be more persistent than the terrestrial forms.

7. Most persistent types are small and inconspicuous forms.

8. Many persistent genera show a slow development, a distinct climacteric period and a long post-climacteric period. Connected with this observation is the other that persistent genera which slowly develop never produce many species during a single geologic period.

9. Minor factors of persistence are seen in (*a*) extreme individual vitality (as in *Lingula* and *Crania*), (*b*) immense broods (as in *Ostrea* and *Limulus*), (*c*) extreme restriction in the matter of food, as in the eaters of carrion and refuse (Capulidæ, oyster, etc.).

The same criteria were found to hold, on the whole, in regard to the persistent species and the higher groups

(families and orders). In the latter case superior sets of offensive arms and defensive armors, early developed, appear to have helped to give stability to some, as in the scorpions (pincers and poison glands), limulids (leathery armor combined with burrowing habit and enormous broods). Some, as the turtles, have successfully specialized for protection.

In trying to reduce the multiplicity of factors to a few controlling agents, it was found " that these are the fixation of the ' over-taken ' and post-climacteric types, the presence of stable physical conditions, and withdrawal in various ways from the fields where the struggle for existence is fiercest. The stable physical conditions have been found by many in the open ocean, by some in the deeper littoral regions of the oceans, by others again in subterranean fields, by some in the rivers and lakes of continental regions that remained undisturbed by folding. Withdrawal from the struggle for existence with other organisms has been accomplished by a variety of means, as by isolation, burrowing life, small, inconspicuous size, superior, often deadly, offensive and strong defensive arms, through restriction to poor fare, great power of endurance, etc."

In an analysis of the biologic factors that have permitted persistence, two entirely different groups of persistent types must be distinguished: (1) The post-climacteric types; (2) the primitive central stocks. The former rely on stable physical conditions and withdrawal from the arena of the struggle for existence, as far as possible; while the latter are frequently dominant in the very seats of war. We have termed the first *persistent terminals,* the others *persistent radicles.*

The persistent terminals were considered to have become so fixed in all their characters as to make them persistent partly by the factors of progressive fixation and partly by the fact that they have in various ways avoided the opposing factor of natural selection; their conservation thus being in fact due in part to their ge-

rontic condition and in part to the peacefulness of their surroundings.

The persistent radicles, on the other hand, were thought to owe their persistence to the fact that through their primitive nature they are still adapted to a greater variety of conditions and that while there may be considerable variation, it is around a still unspecialized, primitive form and thus difficult of recognition.

Or, expressing the same difference in terms of the four processes of heredity, ontogeny, environment and selection, around which, according to Osborn, the life and evolution of organisms continuously center, we found that "the difference between the two groups of persistent types, the relatively rigid terminals and the more variable radicles, consists in the fact that in the former all factors have become fixed and unresponsive to stimuli, only the selection still slowly acting, while the latter are so well adapted to a variety of conditions that no changes readily originate through any of the processes of environment, ontogeny and selection, which affect the whole stock, while at the same time no changes in the germ plasm are induced through hereditary tendencies."

The following notes are written with the intention partly to add certain new factors that appear to contribute to the persistence of forms, and that had not been taken into account in the first essay; and partly to enter deeper into the analysis of the ultimate causes of persistence made possible through more recent investigations into the nature of phylogenesis.

### 1. Additional Factors of Persistence

The new factors here mentioned have all to do with the methods of reproduction whose influence had not been recognized, in the first paper, in the percentage table of persistent genera.

(*a*) *Reproduction by Simple Division.*—In the Protozoa reproduction takes place by division without any

loss, so that there is no distinction between parent and offspring. There is no death and thus it is that Weismann and others have spoken of the "immortality of the Protozoa." It is certainly significant, in this connection, that among the Foraminifera 56 per cent. of the genera were found to be persistent and many were found to exhibit tremendous persistence, ranging from the Ordovician, Silurian, Carboniferous and Triassic to recent times, and that even species (see Ruedemann, *op. cit.* p. 126) are known to extend from Silurian, Devonian, Carboniferous and Triassic times to the present. These forms the writer designated as actual "immortal types" in contrast to the theoretically immortal protozoans of Weismann.

There occurs, however, among the protozoans besides this asexual mode of reproduction a group of processes that are clearly the primitive beginnings of fertilization. In these forms of conjugation different stages may be distinguished, viz., the mere congregation of cells in groups without visible exchange of plasms (cytotropy); the exchange of substance taking place only through osmotic processes; further conjugation, where real fusion of plasmas occurs but the cell-nuclei remain separate (plastogamy); and finally such modes of conjugation, where also nuclear fusion of the conjugating cells takes place (karyogamy); and here again, the pairing cells may be either similar in size (isogamy), or even markedly dissimilar in size (anisogamy).

It is, however, to be remembered that the usual reproductive process among protozoans is simple fusion of ordinary vegetative cells and conjugation as a rule occurs at rare intervals in most forms, often only when unfavorable conditions arise, or as Maupas' experiments indicate, the individuals in the course of numerous successive asexual generations grow old.

(*b*) *Reproduction by Budding.*—This mode of asexual reproduction differs from that of division originally in the protozoans merely in the different sizes of the daughter-

cells and the mother-cell, but develops into a complex process in the multicellular forms. Distinct budding occurs already in the protozoans as in *Arcella,* where a number of small buds are constricted off all round. In sponges it is developed to such a degree that no one can fail to recognize the impossibility of drawing any rigid line between growth and asexual reproduction.[2] In the cœlenterates asexual reproduction runs riot, as Geddes and Thompson state. It is, further, by far the prevailing mode of reproduction among the stock-building bryozoans; it also is common among marine worms, as with the famous palolo-worm off the coast of Samoa, and finally it is also frequently found among the tunicates.

The primitive character of this mode of reproduction can not be doubted. It probably in all cases is an inherited character that persisted from the ancestral protozoans. It has by many zoologists been considered as an acquired character among the tunicates, but Van Name[3] has lately advanced good reasons for the conclusion that it is also a primitive character among the ascidians inherited from their remotest ancestors and that it is not a faculty that can be acquired secondarily.

Budding leads to the formation of colonies or stocks. These as a rule are not favorable to a swimming or vagrant mode of life, hence by far the majority of budding forms are sessile, although there are a considerable number of exceptions in the swimming siphonophores, ctenophores, floating graptolites, and compound swimming worms and ascidians. Since most of the colonial stocks are sessile, budding has often been considered as having been induced by a sessile mode of life and thus held to be a function that could be acquired. Its absence among the sessile cirripedes seems, however, to support Van

[2] Geddes, Patrick, and Thompson, J. Arthur, "The Evolution of Sex," London and New York, 1914, p. 205.

[3] Van Name, Willard G., "Budding in Compound Ascidians and other Invertebrates, and its bearing on the Question of the Early Ancestry of the Vertebrates," *Bull. Amer. Mus. Nat. Hist.,* Vol. 44, art. 15, 1921, pp. 275–282.

Name's contention that this function can not be acquired when once lost.

The fact that the sessile forms contain more persistent types (corals have 15 per cent., bryozoans 22 per cent.) than the vagile benthos would suggest that budding may be a mode of asexual reproduction favorable to the persistence of types; and that it may be the cause of the large percentage of persistent types among the sessile forms. It must here, however, be considered that also the sessile Cirripedia which lack the function of budding, have furnished 20 per cent. of persistent types; and further that in all the classes here considered budding is associated with sexual reproduction, often, as in many cœlenterates, in a regular alternation of generations. Moreover, the sexually reproducing brachiopods, gastropods and pelecypods have furnished large percentages of persistent types, a large number of which are sessile forms.

While thus budding would not seem to be the controlling factor in the persistence of the sessile forms, it is, nevertheless, true that budding may have a distinctly retarding effect upon the evolution of such forms, principally by the material decrease of the cases of sexual reproduction. As in the case of the corals, the number of new stocks that originate from sexual reproduction and finding a new lodging place, start new colonies, is very small when compared with the number of asexually produced individuals on the stocks. There are therefore many more generations of asexually than sexually produced individuals.

(*c*) *Reproduction by Hermaphrodites.*—Another factor that possibly may have contributed to the persistence of forms is hermaphroditism. Claus has pointed out that hermaphroditism finds most abundant expression in sluggish and fixed animals. "Among sponges, sea-anemones, corals, Polyzoa, bivalves, etc., we find frequent illustration of the association of fixedness and hermaphroditism" (Geddes and Thompson, *op. cit.*, p. 83). The origin of

hermaphroditism is still a matter of dispute (see Geddes and Thompson, pp. 83, 84) for while some, as Simon, attribute it to a plethora of nutrition (as especially in parasites), others are " content to interpret it as an adaptation to ensure fertilization, for the possibilities of pairing between separate sexes are certainly lessened if the animals are sluggish, sedentary or parasitic." There is likewise difference of opinion as to whether the stage of hermaphroditism is the lower, and the condition of distinct sexes has been derived from it (Gegenbaur), or whether it is a secondary condition, derived from primitive uni-sexuality as claimed by Pelseneer who considers it grafted on the female sex in Mollusca, Crustacea and Pisces (Geddes and Thompson, p. 84).

Considering its prevalence among the lowest classes with sexual reproduction, notably the sponges and corals, and again among the Cirripedia, we believe that hermaphroditism is in the former an inherited primitive character and in the latter an acquired one. At any rate, since it is so frequently and distinctly associated with sessility, as in the just mentioned Cirripedia, and in many pelecypods (oyster) and with sluggishness in other pelecypods and many gastropods, and since it is exactly these same groups which contain numerous persistent types, it seems probable that hermaphroditism is a further reproductive condition contributory to persistence.

(*d*) ***Reproduction by Parthenogenesis.***—Parthenogenesis is the mode of propagation in at least one typically persistent genus, viz., *Apus;* but it has also become a confirmed physiological habit in other archaic types of crustaceans among the branchiopods, as notably in *Artemia,* the brine-shrimp, in *Branchipus,* and in *Limnadia;* further in the equally primitive water-fleas (*Daphnia* and *Moina*) and finally, among the ancient ostracods, also in some species of the common *Cypris.*

Of the whole class of Branchiopoda, which through paleontology, and notably through the recent amazing

discoveries of Walcott[4] in the Middle Cambrian of British Columbia, are proven to reach back to the oldest fossiliferous beds (in *Protocaris marshi* Walcott to the Lower Cambrian), *Apus* is the most remarkable and most often cited form in paleontologic literature. The writer has in a paper, now in press, shown that true *Apus,* identical in form of carapace and " shell glands " has been found in Permian beds of Oklahoma. It was before known from the Triassic Buntsandstein of the Vogesian Mountains. Its more than 70 pairs of gill-bearing feet and other primitive characters have made it the model of comparison for Paleozoic crustaceans, especially the trilobites. The Lower Cambrian *Protocaris marshi* is so closely allied to *Apus* that it was termed *Apus marshi* by Bernard. There is hence no doubt of the immense age of this type.

*Apus* is now so parthenogenetical in its reproduction that the males were not discovered until a hundred years after the description of the first and best known species (*A. cancriformis* Schäffer); and " von Siebold repeatedly investigated every member of a colony of *Apus,* once over 5,000 in number, without finding a single male. At other times he found one per cent. while in certain unknown conditions (probably when food is scarce and life generally unfavorable) the males may be developed in crowds " (Geddes and Thompson, p. 189). Similar conditions prevail in the brine-shrimp and the other branchiopods, cited above, as shown by Lereboullet and Nowikoff.

Parthenogenesis is associated with other strange habits in the three branchiopods, *Apus cancriformis, Limnadia hermanni,* and *Branchipus stagnalis,* which occur together in Europe. These creatures occur only after very wet seasons in puddles, road-ditches and other small pools, where their eggs have lain for decades in the dry mud, exposed to heat and frost. They develop with amazing

[4] Walcott, Charles D., "Middle Cambrian Branchiopoda, Malacostraca, Trilobita, and Merostomata," Smithsonian Miscellaneous Collections, Vol. 57, No. 6, 1912.

rapidity, *Apus cancriformis* reaching in two weeks a full size up to five inches,[5] produce an enormous number of eggs and die.

The origin of parthenogenesis in these forms as well as in the rotifers and certain insects has been fully discussed by Geddes and Thompson, and they are certain that it has originated as a degeneration from the ordinary sexual process (*ibid*, p. 198) and is no direct persistence of a primitive ideal state. Their theory of parthenogenesis is that the ova that develop parthenogenetically "are to be regarded as incompletely differentiated female cells, which retain a measure of katabolic (relatively male) products, and thus do not need fertilization" (they form only one polar body). "Such a successful balance between anabolism and katabolism is indeed the ideal of all organic life. In parasitic fungi, sexual reproduction disappears, and surrounding waste products presumably help the purpose otherwise effected by sexual organs, so peculiarities in the conditions of parthenogenetic ova may explain the retention of the normal balance which makes division possible without the usual stimulus of fertilization. Abundant and at the same time stimulating nutrition (Rolph), early differentiation of the sex-cells (Simon), the general preponderance of reproductive over vegetative constitution (Hensen), their liberation before the anabolic bias has carried them too far, are among these favoring conditions."

Parthenogenesis thus appears as a degenerative asexual process arising from peculiar conditions, the most important of which appears to be temporary over-nutrition. As in the other asexual modes of propagation, in division and budding, the inference suggests itself readily that this suppression of fertilization must induce persistence, for as Geddes and Thompson point out (*ibid.*, p. 193) the establishment of parthenogenesis and the ab-

[5] See Bruno Weigand, "Mitteilung über das Auftreten der Limnadia Hermanni Ad. Brgt. bei Strassburg im September 1912," *Mitt. der Philomat. Gesellsch. in Elsass-Lothringen*, Bd. 4, Heft. 5, Jahrgang 1912; 1913, p. 730.

sence of fertilization probably involves some diminution in the frequency and range of variability and thus the establishment of parthenogenesis will be a handicap to evolution.

In the case of *Apus*, and its other associated branchiopods as well, it is probable that the successful adaptation to special conditions is a strong contributing factor in the establishment of persistence, as pointed out by the writer in the former paper. It is possible that *Apus* has existed under these conditions from very early times.

Summing up the evidence on persistence of types from the habits of reproduction, it seems that simple division, budding, hermaphroditism and parthenogenesis have each contributed to this persistence and in their way acted as factors that arrested evolution, and that thus help to explain the relatively large percentage of persistent types in the protozoans, sponges, corals, molluscs and the just mentioned branchiopods among the crustaceans.

While the facts thus seem to indicate that these modes of reproduction, other than the normal process of fertilization, were favorable to persistence in fossil types, it is, in the present stage of our knowledge of the meaning of fertilization, not so simple to recognize the underlying cause of their arresting influence on evolution.

The simplest explanation would obviously be to see in fertilization the principal cause of *variation*, as such authors as Treviranus, Brooks, Galton, Weismann and Oscar Hertwig have done. Weismann has insisted that the intermingling of two "germ-plasms" is an important fountain of congenital variation. It can be readily seen that, under this view, the retarding effect of fission, budding and parthenogenesis consists in the exclusion, or restriction to long intervals, of fertilization, thereby reducing variability and the possible action of selection. It is also plausible under this view that mutual fertilization between hermaphroditic individuals tends toward equalization of characters; and this tendency towards equalization is still more increased by fertilization within

the same colonial stock or neighboring colonial stocks of plantations. The most important of the disadvantages resulting from hermaphroditism would then be to reduce the variability which is necessary to progress in the struggle for existence.

While, however, the possibility is not denied that fertilization may be a controlling factor in variation, as stated, *e.g.,* by William E. Kellicott in his "Text-book of General Embryology," 1913, p. 216, it is also obvious, according to the same author, that the evidence for this view is still scanty and uncertain and, moreover, there are two exactly opposed views as to the nature of the relation. While Hertwig maintains that the effect of fertilization is to limit variation within a species, Weismann asserts that the effect of syngamy or "amphimixis" is to cause or promote variation.

Kellicott (*op. cit.,* p. 214) states:

> There is little direct factual evidence for or against these views, either one of which can be maintained upon theoretical grounds. In a few cases it is known that the amount of variability is not significantly different among sexually (gametically) or asexually (parthenogenetically) produced individuals of the same species. And from the standpoint of more recent studies upon heredity and variation the evidence is chiefly either negative or opposed to the idea that this relation constitutes an important element in the origin or present function of fertilization. The present aspects of this relation between fertilization and variation merge in the larger question of the relations with heredity.

While among the higher classes fertilization has become a stimulus to reproduction and a means of heredity, evidence from the lower groups tends to show that fertilization in its results has undergone evolution like every other organic function.

The view is widely accepted today (see Kellicott, p. 209) that among the Protozoa the processes of reproduction and fertilization are not fundamentally related, and the primary significance of fertilization must be sought in some other direction.

The observations made on protozoans have led to the

rejuvenation hypothesis, chiefly represented by Bütschli, Maupas and Richard Hertwig. "It has been found that protoplasmic activity tends gradually to diminish in intensity, and that associated with this diminution are certain morphological alterations in the structure and composition of the cell" (Kellicott, p. 209). These modifications are known as senescence, the senescent condition of the cell consisting frequently in the relatively large proportion of cytoplasm as compared with nuclear substance. Conjugation is assumed to restore the senescent protoplasm to its original condition of vigor, bringing about rejuvenation. It follows from this that protoplasmic activity is cyclic and that periods of senescence would lead to death unless fertilization should occur.

The real evidence for this cyclic character of the life processes of the Protozoans has been furnished by the observations of Maupas and Calkins on *Paramecium*. But observations of Jennings have shown that in different forms of *Paramecium* conjugation and rejuvenation may occur at very different intervals, and Woodruff has been able to prevent cyclic relations by substituting normal conditions for the artificial and more uniform ones of the laboratory. "By continually altering the character of the food, and by imitating in other ways the naturally variable conditions of pond life, he has been able to continue a single race of *Paramecium* for over five years" (quoting from Kellicott), during which period more than 3,000 generations were formed by simple fission. It follows from these observations that protoplasmic activity among the Ciliata may not be cyclic in character under certain conditions, and that when cyclic periods of depression or senescence do occur, the protoplasm may be restored to a condition of normal vigor. either by physical or chemical stimuli, or by fertilization (Kellicott, p. 212).

Fertilization is in these cases a form of reaction that takes place when external conditions become too uniform to bring forth the normal vegetative activities, and that

leads to an internal disturbance, thereby correcting the conditions of uniformity.

Applying these conclusions to our case of the persistent types it could be conceived that the reduction of fertilization to rare intervals, or its entire suppression, in the numerous persistent types that reproduce by fission, budding, parthenogenesis or hermaphroditism, produces a perpetual senescent condition that while not leading to death as in the rapidly dividing and sensitive Paramecium, finds its expression in the rigidity of the forms, recognizable in their lack of response to external stimuli and of further evolution, *i.e.,* in their persistence. Or in other words, infrequency or entire lack of rejuvenation through fertilization favors the persistent condition, at least among those persistent terminals that do not live under stable physical conditions. Those living under stable conditions may require fertilization as a necessary rejuvenating process counteracting progressive senescence and final extinction through lack of external stimuli. It would then appear that these lower modes of reproduction and very stable external conditions could not very well exist together.

However, as pointed out by Kellicott, there has been an evolution both of the process and of the consequences of fertilization, and the various possibilities as to the significance of fertilization are not mutually exclusive. It is therefore possible that the large percentage of persistent types among forms with more or less suppressed fertilization finds its explanation in some cases in the resulting lack of variation, in others in the resulting senescent and rigid condition of the race, and in still others it may be sought in the process of heredity, connected with fertilization. This last possibility will be dealt with in the following chapter.

### Reduction of Factors to Fundamental Causes

The investigation of the various groups of persistent types has indicated that there are a variety of factors

involved in their production. Many of these were found to be connected with the environment, others, acting through variability, or its lack, with selection, and still others with the processes of heredity and ontogeny. While of these four fundamental processes of evolution, viz., heredity, ontogeny, environment and selection, that of selection may account for the cases of persistence where variability has been reduced to a minimum, possibly by the lower modes of propagation mentioned above, and that of environment accounts for persistence in those cases where the environment has become so stable as to lack the actual stimulus for further development, it is obvious that still more important factors are involved in heredity and ontogeny that make for persistence in organisms, especially as it is seen in the post-climacteric forms, or persistent terminals. Both the conservative process of heredity and the much less rigid one of ontogeny appear to become more or less fixed and inaccessible to changes in persistent types.

None of these four processes gives any clue to the actual mechanics of the factors that induce persistence. In trying to trace the latter to its ultimate causes, it becomes, therefore, necessary to go beyond these processes, and to appeal to the important conclusions that have been obtained by modern experimentation and observation regarding the methods of inheritance and production of new characters by means of the genes or character-determiners of the heredity-chromatin.

Among these conclusions especially suggestive in regard to our problem, are the views advanced by Dürken and Salfeld.[6] These authors have, one through an analysis of all recent zoological experiments on evolutionary problems, the other through a corresponding analysis of the evolution among the fossil ammonites, arrived at the view that variability or the appearance of new characters, and of new combinations of characters is produced in differ-

[6] Dürken, B., and Salfeld, H., "Die Phylogenese. Fragestellungen zu ihrer exakten Erforschung," Berlin, Gebr. Borntträger. 1921.

ent ways, by the genes; and not only through internal factors, as claimed by the Neo-Darwinian school, but also through external ones as demanded by the Neo-Lamarckians. The genes, which are not only actual units, or representatives of definite phenotypic characters, but definitely delimited, material bodies, may not only produce new characters or character-combinations by a correlative and a combinative mode of ontogenetic evolution, or by loss of genes, as demonstrated by abundant experiments, but undoubtedly there takes place also a new formation of genes in evolution. This they hold to come about in successive stages through long enduring external influence, which first acts upon the cytoplasm of the cells and especially of the germ-cells. This cytoplasm in itself has been proven to have certain hereditary possibilities (plasmogenous heredity). Under long persistent external influence there form first preliminary stages of genes in the cytoplasm which finally, when a certain "threshold" (Schwelle) of continued strain is passed, become true genes of the heredity-chromatin. When this takes place, mutations appear abruptly (salto-mutations).

This view, here altogether too briefly presented, would explain the absence of evolution through salto-mutations in cases of persistence under continued stable exterior conditions, and since the cytoplasm is known also to influence directly the heredity-chromatin, also the absence of fluctо-mutations or variations under stable conditions through lack of external stimulation.

However, in the cases where no new genes are formed by external influences, new characters could still appear through loss of genes or correlative or combinative modes of production of new genes from the old ones within the germ-plasm. This, however, leads to a restrictive cone of divergence ("Streuung") of the characters and through "self-differentiation" by a combinative mode of gene-production to the excessive characters of many terminal series (*e.g.*, dinosaurians); and to the rigid

persistent terminal types, on the other hand, through the gerontic rigidity of the remaining stock of genes. The principal causes of the persistence of terminal forms would then be the failure of production of new genes arising from the cytoplasm, through external influences, and the senescent rigidity of the remaining genes.

The persistent radicles, on the other hand, correspond to the extreme development of what Salfeld terms "Konservativreihen." There are series in which the salto-mutations appear in very long intervals, while the numerous side-branches (which furnish the index-fossils) develop by rapid salto-mutations. These persistent radicles are therefore able to undergo new periods of explosive and climacteric development ("Virenz-perioden" of Wedekind) and are thus still less absolutely persistent than the persistent terminals. In these conservative series, according to Salfeld, flucto-mutation is so prevalent that sharply defined "species," or better mutants, can not be separated, as notably in the phyla of Phylloceras and Lytoceras which range, qualitatively unchanged in their characters, through Jurassic and Cretaceous time. They thus represent true persistent radicles. This fact, combined with the observation of the vitality, relative primitive simplicity and adaptation to a variety of conditions of persistent radicles, pointed out by the writer in his former paper, suggests that the complex of genes is able to remain relatively undisturbed through external influences (only flucto-mutations appearing) in one part of these groups which persist as radicles, while those parts which become changed through the addition of genes by way of the cytoplasm turn into the side-branches by salto-mutation.

# EXPERIMENTAL STUDIES ON THE DURATION OF LIFE

## III. The Effect of Successive Etherizations on the Duration of Life of Drosophila[1]

PROFESSOR RAYMOND PEARL AND SYLVIA L. PARKER

### Purpose and Plan of Experiments

In any experimental work of a genetic character on *Drosophila,* it is often necessary to anesthetize the flies which are to be used in an experiment for a sufficiently long time so that they may be sexed and sorted into different groups for the purpose of making matings, etc. It has been shown by Morgan (33) that this procedure has no effect upon the causation of morphological mutations, the inheritance of which he has studied (9). The effect might, however, conceivably be quite different in the case of a physiological character like duration of life. Any one who has undergone a major surgical operation feels that anesthetization is at least immediately a rather profound physiological disturbance. Unfortunately, so far as we are aware, no accurate determinations have ever been made to show whether in man one or more anesthetizations changes the expectation of life. As a matter of fact, there are presumably no human data on the point available in any such amount as would be necessary for actuarial determinations, because in man anesthetization is, generally speaking, only undertaken in connection with surgical operations of greater or less severity, so that if we did have statistics of expectation of life of persons who had been anesthetized, there would always be involved the two factors of anesthetization and oper-

[1] Papers from the Department of Biometry and Vital Statistics, School of Hygiene and Public Health, Johns Hopkins University, No. 54. For description of the method of numbering bibliographic citations see the second paper in the series (32).

ation. In *Drosophila* these two factors can be separated.

It has seemed important, in an early stage of our experimental work on the duration of life in this form, to make a careful and extensive experimental test of the question of whether anesthetization singly or repeated changed in any way the expectation of life or form of the life curve, so that if this factor does have any significant influence, either favorable or unfavorable, due allowance may be made for it. It is the purpose of this paper to report the results of such a test.

The flies used in this experiment were flies of line 107 (generation 8 since January 14, 1921, line bred from a single brother and sister mating for approximately 30 generations). The characteristics of this line relative to duration of life have already been described (*cf.* Pearl and Parker (32)). The 4,330 flies used emerged between 10 A.M. April 18, 1921, and 4 P.M. April 22, 1921, from thirty-five mass cultures started in half-pint milk bottles April 7, 1921. The regular procedure in these experiments was to collect the flies from all 35 breeding bottles in one empty bottle and then to count the flies through a counting tube into 1-ounce vials, allowing 50 flies to each vial.[2] Ten vials were used for each series, except the control series, which had 18. For two of the series only two hours were allowed between successive emptyings of the mating bottles, to get flies at an average age of one hour, assuming that they emerged uniformly over the interval. One series was etherized as soon as counted out, and the other series kept as a special control group to see if the handling when the flies were so young and soft had any effect on the duration of life. For the rest of the series the flies were allowed to emerge over a 24-hour interval. Each day's hatch was divided randomly and as equally as possible among the different series.

[2] It will be noted that the totals shown in Table I do not accord exactly with this statement. The discrepancies are due to the fact that a few flies were lost in changing to fresh bottles in the course of the life duration determinations made according to the technique described in (27), and occasionally a bottle was broken by accident and all its contained flies lost.

The counting tube referred to above is a device invented in this laboratory which we find extremely useful in a great deal of the experimental work. It was devised and first used in connection with studies of the growth of experimental populations of *Drosophila* (*cf.* Pearl (7), and Pearl and Kelly (34)). Its construction is shown in Fig. 1.

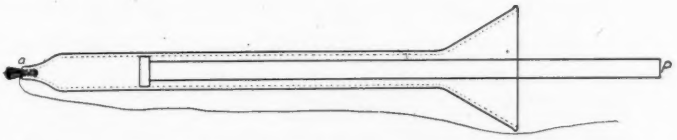

FIG. 1. Diagram showing construction of *Drosophila* counting tube. The aperture at *a* is just large enough to allow one fly to pass through at a time. The essential dimensions are as follows: length over all 25 cm., diameter of main tube 2 cm., diameter of funnel mouth 6 cm.

When it is desired to count a definite number of flies the small aperture *a* is temporarily plugged with a bit of cotton wool, the plunger *P* is removed from the tube and flies are shaken into the counting tube by inverting the open bottle containing them over the funnel mouth of the counting tube. Then the plunger is inserted and gently moved forward to concentrate the flies in the lower end of the counting tube. Then the counting tube with enough cotton around it to close up the mouth of the bottle is inserted into the bottle into which it is desired to place the counted flies and the plug removed from the aperture *a*. Then as the flies come out of the tube, one by one, through the aperture *a*, they are counted as they pass this point, with the aid of a tally register, such as is used by doorkeepers at theaters, etc. The plunger is gently moved forward as necessary to keep up an even flow of flies through the mouth of the tube.

The ether dose used was constant for all the flies throughout the experiment. The group to be etherized was shaken into a clean half pint milk bottle; 5 c.c. of ether was poured onto a piece of absorbent cotton fastened to the under side of a cork stopper; the bottle with

the flies was stoppered tightly with the cork and left for two minutes. Then the flies were turned out on a tile and sexed and counted (since that operation corresponds in extent of handling to what we need to do in making up matings, etc.), then emptied into a vial with fresh food, where they recovered from the ether in about half an hour. For each successive group of flies a fresh bottle and fresh cotton for the ether were of course used.

In all other, here unspecified, particulars the technique used in these ether experiments was uniformly that described in detail in the first paper of this series (27).

Seven series of experiments were conducted, differing in respect of the number of times the flies were etherized, and in their age at the time of etherization. The seven series were as follows:

*A*. Etherized once when one hour of age.
*B*. Etherized once when twelve hours of age.
*C*. Etherized once when thirty-six hours of age.
*D*. Etherized once when three and a half days of age.
*E*. Etherized twice when seven, and fourteen days of age, respectively.
*F*. Etherized three times when seven, fourteen, and twenty-one days of age, respectively.
*G*. Etherized four times when seven, fourteen, twenty-one and twenty-eight days of age, respectively.

## DATA

The $l_x$ lines for the several series of etherized flies and the controls are given in Table I. These $l_x$ distributions are calculated on the basis of 1,000 flies at emergence from the pupal stage, with the absolute number of flies on which the distribution is based given at the bottom of the column in each case.

The $l_x$ distributions for all etherized flies and for their controls in the ether experiment, and for two tests of the flies in line 101 and its continuation 107, are shown graphically in Fig. 2. The data for the survivorship lines in the two tests of line 107 are to be found in Pearl and Parker (27).

TABLE I

SURVIVAL DISTRIBUTION OF ETHERIZED AND NON-ETHERIZED DROSOPHILA CULTURES

| Age in Days | Etherized Series | | | | | | | | All Controls | Controls 1 hr. Old | Controls 12hrs Old |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | *A* | *B* | *C* | *D* | *E* | *F* | *G* | All Etherized | | | |
| 1– 6 | 1,000 | 1,000 | 1,000 | 1,000 | 1,000 | 1,000 | 1,000 | 1,000 | 1,000 | 1,000 | 1,000 |
| 7–12 | 988 | 998 | 988 | 998 | 993 | 990 | 995 | 993 | 985 | 990 | 983 |
| 13–18 | 986 | 993 | 978 | 995 | 984 | 983 | 995 | 988 | 980 | 987 | 976 |
| 19–24 | 984 | 971 | 978 | 984 | 971 | 971 | 964 | 975 | 974 | 981 | 970 |
| 25–30 | 970 | 953 | 966 | 965 | 957 | 918 | 910 | 949 | 925 | 918 | 928 |
| 31–36 | 942 | 916 | 912 | 942 | 944 | 910 | 857 | 918 | 903 | 895 | 907 |
| 37–42 | 902 | 880 | 856 | 926 | 901 | 879 | 810 | 880 | 854 | 843 | 859 |
| 43–48 | 778 | 722 | 796 | 847 | 793 | 763 | 698 | 771 | 735 | 753 | 724 |
| 49–54 | 629 | 634 | 732 | 725 | 643 | 598 | 605 | 652 | 604 | 701 | 550 |
| 55–60 | 453 | 492 | 625 | 581 | 465 | 462 | 476 | 507 | 454 | 554 | 398 |
| 61–66 | 117 | 95 | 397 | 301 | 153 | 249 | 145 | 183 | 150 | 138 | 157 |
| 67–72 | 47 | 34 | 136 | 116 | 79 | 165 | 71 | 92 | 53 | 71 | 43 |
| 73–78 | 12 | 9 | 29 | 37 | 22 | 65 | 17 | 27 | 15 | 19 | 13 |
| 79–84 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Absolute number of flies.... | 428 | 443 | 411 | 432 | 445 | 413 | 420 | 2,992 | 1,338 | 478 | 860 |

It is at once evident, from an examination of the figures in Table I, and the diagram, that there was no considerable difference in duration of life, or in the form of the life curve, for the etherized flies taken as a class, and the non-etherized groups. It is, however, desirable to examine the results of the experiments in detail in order to see whether there are detectable by biometric methods any small but still statistically significant differences between the several groups. To this end, Table II has been prepared, giving the usual biometric constants for the several series.

Comparing first the entire etherized group as a whole with those which never had any ether at all in their lives, it is seen that the mean duration of life (expectation of life at emergence from pupa) is $1.82 \pm .30$ days longer in the former (etherized) than in the latter (normal) group. The difference is thus slightly more than 6 times

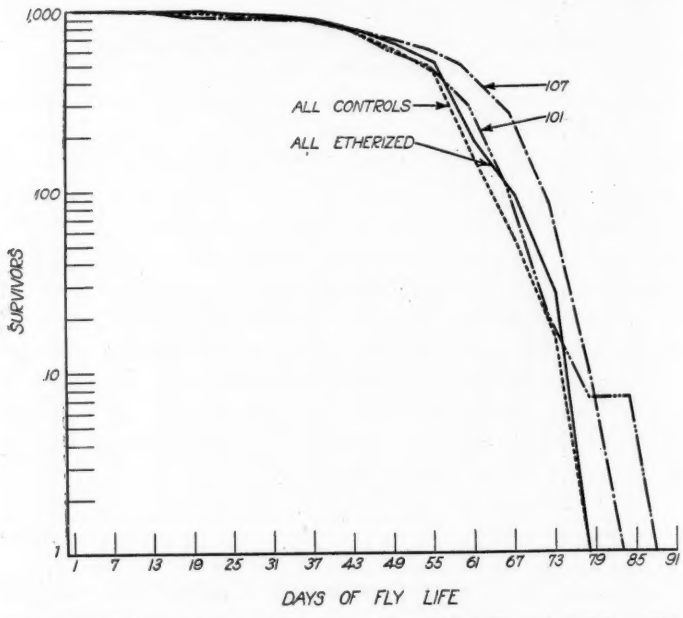


FIG. 2. The $l_x$ lines for all etherized and controlled flies, plotted from the data of Table I.

TABLE II

BIOMETRIC CONSTANTS FOR DURATION OF LIFE OF ETHERIZED AND NORMAL DROSOPHILA

| Treatment | Number of Flies | Mean (in days) | Standard Deviation (in days) | Coefficient of Variation |
|---|---|---|---|---|
| All etherized | 2,992 | 51.60 ± .16 | 13.30 ± .12 | 25.77 ± .24 |
| All controls | 1,338 | 49.78 ± .25 | 13.68 ± .18 | 27.47 ± .38 |
| Etherized when 1 hour old | 428 | 50.82 ± .38 | 11.76 ± .27 | 23.14 ± .56 |
| Etherized when 12 hours old | 443 | 50.20 ± .39 | 12.25 ± .28 | 24.41 ± .59 |
| Etherized when 36 hours old | 411 | 53.36 ± .46 | 13.91 ± .33 | 26.06 ± .65 |
| Etherized when 3½ days old | 432 | 54.50 ± .40 | 12.36 ± .28 | 22.68 ± .55 |
| Etherized when 7 and 14 days old | 445 | 51.43 ± .40 | 12.50 ± .28 | 24.31 ± .58 |
| Etherized when 7, 14, and 21 days old | 413 | 51.72 ± .50 | 15.01 ± .35 | 29.03 ± .74 |
| Etherized when 7, 14, 21, and 28 days old | 420 | 49.26 ± .47 | 14.42 ± .34 | 29.26 ± .74 |
| Controls (taken out of mating bottles when 1 hour old) | 478 | 51.11 ± .42 | 13.75 ± .30 | 26.90 ± .63 |
| Controls (taken out of mating bottles when 12 hours old) | 860 | 49.04 ± .31 | 13.58 ± .22 | 27.69 ± .48 |

its probable error, and must therefore be regarded as statistically significant. Absolutely, however, the difference is small. It is equivalent to only 3.7 per cent. increase of the expectation of life of the controls. In variability in respect of duration of life there is plainly no significant difference between etherized and control groups.

It is not entirely clear that the small difference between the etherized and control groups in mean duration of life can be regarded as due to the influence of the ether. An examination of the last two lines of Table II shows that an entirely similar difference in the means appears between the two control groups, which differ only in respect of the time when they were taken from the mating bottles, and without either having been etherized. The difference in these two means amounts to $2.07 \pm .52$ days, a statistically significant and absolutely slightly larger difference than that between etherized and control groups. Again there is no significant difference in variability in the two groups.

Altogether we shall be justified in concluding that there is no evidence from these experiments that the occasional etherization of *Drosophila* to the extent necessary in sexing and making matings alters the expectation of life by an amount large enough to introduce any sensible source of error into experiments on the duration of life in this form, except possibly where the most careful and accurate actuarial determinations need to be made. Then it will be well to have this possible source of error in mind and to plan the experiments in such way as to check it.

Examining the results for the different etherized series it is seen that the highest mean duration of life appears in the group etherized once at $3\frac{1}{2}$ days of age, and next to this stands the group etherized once at $1\frac{1}{2}$ days of age. Both of these give relatively high mean values. There also appears a definite, though not particularly marked tendency for the variability in duration of life to be greater in the groups which were etherized several

times. No great importance is probably to be attached to these differences between the several groups, however, though some of them appear significant statistically.

## CONCLUSION

From the experiments herein described, involving the determination of the total duration of life in 4,330 individual flies, it may be safely concluded that no sensible error will be introduced into duration of life experiments on *Drosophila* as a result of completely anesthetizing the flies with ether, at least up to as many as four times in the course of their lives.

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## SHORTER ARTICLES AND DISCUSSION

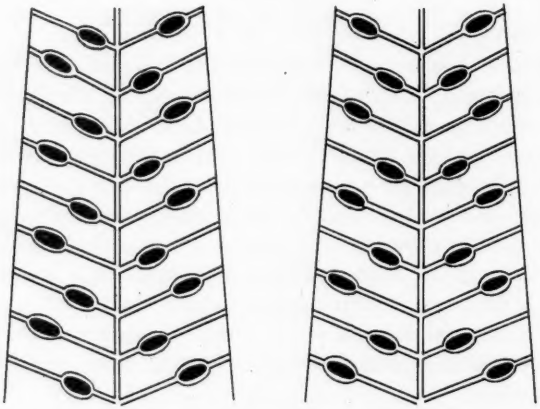
### A TEACHING NOTE ON THE ARRANGEMENT OF THE TUBE-FEET IN ASTERIAS

SEVERAL summers ago while directing the laboratory work on Echinodermata in the Invertebrate Course at Woods Hole, a question was raised by one of the students as to the correctness of the account which had been given of the arrangement of the tube-feet in the common starfish, *Asterias forbesi* Desor. An examination of the point in question revealed the occurrence of a rather interesting irregularity which is here briefly reported on. The source of the conditions here described is entirely unknown, but inasmuch as this starfish is commonly used as material for laboratory study, it occurred to me that the facts themselves might be of interest to teachers of invertebrate zoology.

It will be recalled that in *Asterias* the tube-feet are arranged in four longitudinal rows, two of which are on each side of the radial canal (the mid-ventral line of the arm). The tube-feet in these rows of two are arranged in an alternate manner, nearer or farther from the mid-line, thus allowing for the accommodation of more tube-feet in a given linear space. The tube-feet are connected with the radial canal by short transverse canals, which are thus longer or shorter according as they pass to tube-feet in the inner or outer series. This arrangement of the tube-feet can be clearly made out in properly dried specimens from which the remnants of the tube-feet themselves are all removed. Their position is clearly marked in such a preparation by the perforations between each pair of ambulacral ossicles, and the whole topography of the ambulacral groove is well demonstrated. It is usually stated that[1] "Each pair of transverse canals consists of a short canal on one side and a longer canal on the opposite side of the radial canal. The short and long canals of each side are alternating." This arrangement of the tube-feet is shown in a diagrammatic way in Fig. 1, in which the tube-feet are represented as black ovals situated in the perforations be-

[1] Quoted from Petrunkevitch, "Morphology of Invertebrate Types," New York, 1916, pages 177 and 178.

tween adjacent ambulacral ossicles. This, the common arrangement, may be designated Type I. It will be noted that as one runs along the arm the transverse canals of succeeding pairs are long-short, short-long, and so on.



FIGS. 1 AND 2

However, it appears from my experience in this laboratory that some teachers give a different description of the arrangement of the tube-feet. According to these teachers the length of the transverse canals does not alternate in a single pair, but is the same on both sides of the radial canal. This would lead to an arrangement which is shown diagrammatically in Fig. 2. According to this account as one runs along the arm the transverse canals of succeeding pairs are long-long, short-short, and so on. It seemed worth while from a teaching standpoint to determine which of these descriptions is the correct one. For convenience, the arms of the starfish will be named in the conventional manner *a, b, c, d, e*—*a* being the first arm to the right of the madreporic plate (as seen from the aboral surface), the others being named in a clock-wise direction around the disc.

In all, seventeen specimens of *Asterias forbesi* have been examined, some more completely than others. The first two or three pairs of tube-feet at the very base of the arm are usually rather crowded by the abrupt narrowing of the ambulacral groove, so that it is rather difficult to say exactly to which type

of arrangement they belong. They seem usually to be more like Type II than Type I. The groove widens rapidly, however, and the four characteristic rows are quickly established. In the majority of cases the arrangement is undoubtedly like Type I, and this is obviously the source of the usual text-book description.

However, in at least nine specimens of the seventeen examined, one or more arms have the Type II arrangement. This may occur in any arm, but in my specimens is most frequent in arm *e* (five cases). Sometimes the Type II arrangement is established from the very beginning of any regularity at the base of the arm; in my specimens there were three (probably four) cases of this kind in arm *e* and one in arm *d*. In such cases the Type II arrangement may persist throughout the entire length of the arm. More commonly, however, the Type I arrangement is first established and after persisting for a longer or shorter distance abruptly changes to Type II. The number of Type I pairs in such cases seems usually to be small. The transformation is made by a slight irregularity on one side such that two long or two short transverse canals are adjacent—and thereafter the arrangement is again entirely regular. Sometimes the region of change is more irregular, but never strikingly so. In one case, in arm *a*, the arrangement was first like Type I, soon changed to Type II, and in the distal part of the arm changed back again to Type I. In the seventeen specimens examined the Type II arrangement has been found to occur (in some part of the arm) as follows:

| | |
|---|---|
| Arm *a* | 3 cases |
| " *b* | 3 cases |
| " *c* | 2 cases |
| " *d* | 1 case |
| " *e* | 5 cases |

The number of arms with Type II arrangement in any one individual varies considerably, the results for my specimens being as follows:

| | |
|---|---|
| 1 arm affected | 7 cases |
| 2 arms affected | 1 case |
| 5 arms affected | 1 case |

In two cases, both arm *a*, Type II was found to occur in regenerating arms, though near the base the Type I arrangement

occurred. Whether the injury to the arm was the source of the change is not apparent.

It will be seen, therefore, from the above account that both descriptions of the tube-feet arrangement are correct, but that the one usually given in text-books (Type I) is by far the more common; furthermore, that the one type may change to the other with no apparent structural reasons for the transformation.

The facts here presented furnish, I believe, a complete explanation of the difference in the laboratory accounts as given by different teachers.

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## THE MICRO-FILTER FOR MINUTE FLAGELLATES

IT is frequently desirable during the study of the minuter protozoa, and especially of the small flagellates, to concentrate the organisms. This the writer has been able to do in a very simple and satisfactory manner by means of the device shown in Fig. 1, which may be called the micro-filter; a name applied not only because of its office, but also because of the minute piece of filter-paper used.

The contrivance consists of a standard, either of wood or of metal, which supports a burette tube, a minute circle of filter-paper, and a vessel beneath. The water containing the protozoa to be concentrated is introduced into the burette from above, by means of a funnel, and the pinch cock ($O$) opened sufficiently to allow the liquid to drop into the small funnel or circle of filter-paper beneath ($P$). The filter is supported by means of stout copper wire. The flow of water from the burette can be nicely regulated by means of the pinch cock, which, to give the best results, should be of the screw variety. The water drops through a glass tube, drawn out into a fine point ($T$). It was found convenient to have several of these tips of different diameters.

Considerable experimentation is necessary before the exact balance between the flow of water from the burette and that from the base of the filter-paper funnel can be secured. When this balance is reached, the burette is filled and the water allowed

to filter into the vessel on the base of the stand. It is necessary, at approximately fifteen-minute intervals, to thrust into the burette, as far down as the shoulder, or point of taper (just above the rubber tube on which the pinch cock rides), a straight

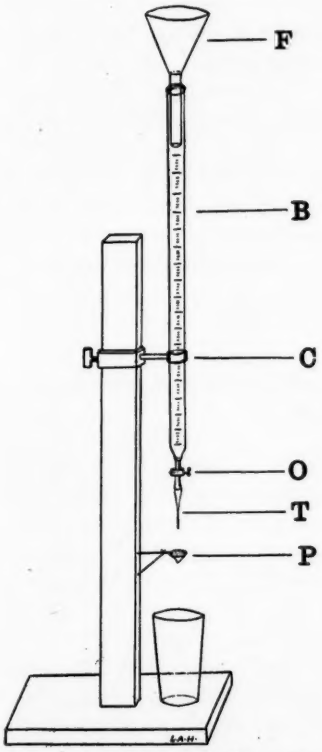


FIG. 1. The Micro-filter. Simple wooden stand for the micro-filter, supporting: funnel (*F*), burette (*B*), clamp for holding burette (*C*), pinch cock (*O*), capillary tip (*T*), filter paper (*P*), and vessel for catching filtered water beneath.

FIG. 2. Pipette with flattened tip for scraping filter paper, to remove filtered organisms.

copper wire rod, holding in its lower end a bit of cotton. This serves to stir up the material which it is desired shall be deposited upon the filter paper, to prevent it from settling and adhering to the sides of the glass, on the slopes of the taper.

When the entire amount of water has passed through the filter-paper, the latter is removed, spread out, and immersed in a bath of water, in a watch crystal. The water should just cover the filter-paper.

The device shown in Fig. 2 is now brought into play. This consists of a glass pipette, flattened and spread at its tip, and serves admirably for gently scraping and sucking the surface of the filter-paper, as it lies in the watch crystal. This withdraws into the pipette the organisms which have been filtered out. These can now be transferred to a glass slip and examined under the microscope, or injected into culture media as inoculations.

The writer has found that, with practice, the possibilities of the micro-filter may be extended to aid, in many ways, in the study of the protozoa.

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## COMPLETE LINKAGE IN *DROSOPHILA MELANOGASTER*[1]

IN 1917 a mating appeared in the cultures of the authors, the flies from which showed no crossing over in the region scute to forked of the sex chromosome, although the factors echinus, cut, vermilion and garnet, were between the extreme points. This culture appeared spontaneously; selection played no part in it. The stock from this culture has now passed through not less than 80 generations and numbers over 3,000 matings. During this time no crossing over has appeared within the known length of the sex chromosome.

In experiments including the second chromosome points, black and purple, it has been shown that no crossing over takes place between these points when complete linkage exists for the first chromosome. Likewise the third chromosome points, dicheate and hairless, have shown complete linkage when the points scute to forked in the first chromosome, and the points black to purple in the second chromosome show the same phenomena.

The disturbing cause is genetic, behaving as a recessive. Its

[1] Papers from the Biological Laboratory, Maine Agricultural Experiment Station, No. 142.

position is in the region of dicheate hairless of the third chromosome. It may be noted that such recessive factors effecting the mechanism of segregation show what might be called delayed Mendelian results for the $F_2$ flies must be tested for their linkage relations before anything can be said regarding the stock.

Complete linkage has been reported in but one other case. Thus in 1912 Morgan showed that crossing over did not occur in the second chromosome of the male of this same species, *melanogaster*. This phenomenon has since been extended to include the other chromosomes. If it be considered that crossing over as originally discovered for the female of this species is the normal, then Sturtevant has shown not less than three dominant factors to materially reduce the normal amount of a crossing over in the second and third chromosomes. A further incompletely analyzed case of the same investigator suggests that a third chromosome dominant partly controls an increase in crossing over in the second chromosome. Crossing over variations have been shown by Bridges in his "deficiency" case, etc. From this it appears that there are three kinds of effects shown by the crossover mechanism. The first case, that of Morgan, shows no crossing over in the male. No genetic factors have as yet been shown to be responsible for this. The second case, that of Sturtevant, shows genetic dominant factors responsible for reducing crossing over in the female. The third case, given here, shows recessive genetic causes allowing no crossing over in the female. It further shows these factors capable of acting on chromosomes of which they are not a part.

Detlefesen and Roberts using the sex-linked factors, white and miniature, present another kind of evidence. In a selection experiment they show crossing over to decline from the normal amount (about 33 per cent.) to nearly zero per cent., no evidence being presented as to the causative agent, although the suggestion is made that "crossing over in the various regions of the sex chromosome (and the other chromosomes?) is probably controlled by multiple incompletely dominant factors." From what has been indicated above it seemed more probable that recessive factors, perhaps one, are responsible for these linkage variations. Especially is this true of their results in series $A$ and $A^1$, for with delayed Mendelian segregation, recessive autosomal factors effecting crossing over in the

sex chromosome, mass mating in every other generation, and complications resulting from only being able to test the female, it is to be expected that selection will progress slowly at first and come suddenly to the climax of reduced crossing over.

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